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FILE 'CAPLUS, BIOSIS' ENTERED AT 15:00:36 ON 19 MAR 2001
L1 1214 S CATIONIC (A) LIPID
L2 972 S CATIONIC (A) LIPOSOME
L3 972 S CATIONIC(A)LIPOSOME
L4 568 S DNA (S) L1
L5 366 S DNA (S) L2

=> d l4 200 ibib

L4 ANSWER 200 OF 568 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:174284 CAPLUS
DOCUMENT NUMBER: 128:305169
TITLE: DNA at membrane surfaces: an experimental overview
AUTHOR(S): Safinya, Cyrus R.; Koltover, Ilya; Raedler, Joachim
CORPORATE SOURCE: Materials and Physics Departments, Biochemistry and Molecular Biology Program, University of California, Santa Barbara, CA, 93106, USA
SOURCE: Curr. Opin. Colloid Interface Sci. (1998), 3(1), 69-77
CODEN: COCSFL; ISSN: 1359-0294
PUBLISHER: Current Chemistry
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

=> d l4 250 ibib

L4 ANSWER 250 OF 568 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:130043 CAPLUS
DOCUMENT NUMBER: 126:127859
TITLE: Use of biologically active peptides to increase the efficiency of transformation with **DNA**:

cationic lipid complexes

INVENTOR(S): Hawley-Nelson, Pamela; Lan, Jianqing; Shih, Pojen; Jessee, Joel A.; Schifferli, Kevin P.

PATENT ASSIGNEE(S): Life Technologies, Inc., USA; Hawley-Nelson, Pamela; Lan, Jianqing; Shih, Pojen; Jessee, Joel A.; Schifferli, Kevin P.

SOURCE: PCT Int. Appl., 64 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 4

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9640961	A1	19961219	WO 1996-US8723	19960604
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9659792	A1	19961230	AU 1996-59792	19960604
EP 874910	A1	19981104	EP 1996-917118	19960604
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI				
JP 11506935	T2	19990622	JP 1996-501227	19960604
PRIORITY APPLN. INFO.:			US 1995-477354	19950607
			WO 1996-US8723	19960604

=> d l4 225 ibib

L4 ANSWER 225 OF 568 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:568307 CAPLUS

DOCUMENT NUMBER: 127:230340

TITLE: Complexes of DNA, cationic lipids and membrane-active peptides for introduction of DNA into higher eukaryotic cells

INVENTOR(S): Wagner, Ernst; Mechtler, Karl; Kichler, Antoine

PATENT ASSIGNEE(S): Boehringer Ingelheim International G.m.b.H., Germany; Wagner, Ernst; Mechtler, Karl; Kichler, Antoine

SOURCE: PCT Int. Appl., 62 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9730170	A1	19970821	WO 1997-EP649	19970213
W: CA, JP, MX, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
DE 19605548	A1	19970904	DE 1996-19605548	19960215
CA 2246227	AA	19970821	CA 1997-2246227	19970213
EP 900281	A1	19990310	EP 1997-904426	19970213
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000504579	T2	20000418	JP 1997-528984	19970213
PRIORITY APPLN. INFO.:			DE 1996-19605548	19960215
			WO 1997-EP649	19970213

=> d 14 210 ibib

L4 ANSWER 210 OF 568 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:34021 CAPLUS
DOCUMENT NUMBER: 128:176641
TITLE: Introduction of a disulfide bond into a **cationic lipid** enhances transgene expression of plasmid **DNA**
AUTHOR(S): Tang, Fuxing; Hughes, Jeffrey A.
CORPORATE SOURCE: Department of Pharmaceutics, University of Florida, Gainesville, FL, 32610, USA
SOURCE: Biochem. Biophys. Res. Commun. (1998), 242(1), 141-145
CODEN: BBRCA9; ISSN: 0006-291X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

=> d 14 220 ibib

L4 ANSWER 220 OF 568 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:644939 CAPLUS
DOCUMENT NUMBER: 127:322701
TITLE: Characterization of cationic liposome-mediated gene transfer in vivo by intravenous administration
AUTHOR(S): Song, Young K.; Liu, Feng; Chu, Shaoyou; Liu, Dexi
CORPORATE SOURCE: Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA, 15261, USA
SOURCE: Hum. Gene Ther. (1997), 8(13), 1585-1594
CODEN: HGTHE3; ISSN: 1043-0342
PUBLISHER: Liebert
DOCUMENT TYPE: Journal
LANGUAGE: English

=> d 211-220 abs ibib

L5 ANSWER 211 OF 366 BIOSIS COPYRIGHT 2001 BIOSIS
AB The p53 gene plays an important role in the regulation of cell-cycle progression and apoptosis. Recent studies have implicated p53 in determining cell fate, and shown that p53 status is associated with cellular sensitivity to anticancer agents. However, the role of p53 in paclitaxel- induced cytotoxicity remains unclear. Here we show that the induction of exogenous wild-type (wt) p53 genes in p53-null human NSCLC H358 cells via transient gene transfection with **cationic liposome**-wt p53 complexes resulted in a typical senescence-like phenotype. In short, cell growth was reduced, homeostasis occurred, cell morphology became enlarged and flat, the cell cycle was arrested at G1 phase, cyclin B1 and cdc2 expression was down-regulated, and **DNA** synthesis was suppressed. The sensitivity of wt p53-transfected cells (H358/p53) to paclitaxel was apprx3-fold lower than that of H358 cells. Paclitaxel treatment gradually and significantly blocked cell-cycle progression at G2/M phase and increased the accumulation of cyclin B1 and cdc2 in H358 cells. In contrast, the same treatment slightly arrested the cell cycle at G2/M phase and slightly elevated cyclin B1 expression in H358/p53 cells. The rate of uptake and efflux of paclitaxel was not significantly different between H358 and H358/p53 cells, indicating that the reduction in cellular sensitivity caused by p53 transfection was not due to alterasion in intracellular drug concentration. Together, our

findings suggest that the induction of exogenous wt p53 gene expression in cells lacking p53 function can trigger the senescence program and that loss of sensitivity to paclitaxel by p53-transfected cells may be associated, at least in part, with the induction of a senescence-like phenotype.

ACCESSION NUMBER: 2000:275830 BIOSIS
DOCUMENT NUMBER: PREV200000275830
TITLE: Induction of senescence-like phenotype and loss of paclitaxel sensitivity after wild-type p53 gene transfection of p53-null human non-small cell lung cancer H358 cells.
AUTHOR(S): Ling, Yi-He; Zou, Yiyu; Perez-Soler, Roman (1)
CORPORATE SOURCE: (1) Kaplan Comprehensive Cancer Center, 550 First Avenue, New York, NY, 10016 USA
SOURCE: Anticancer Research, (March April, 2000) Vol. 20, No. 2A, pp. 693-702. print..
ISSN: 0250-7005.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

L5 ANSWER 212 OF 366 BIOSIS COPYRIGHT 2001 BIOSIS
AB It is shown that calcium increases the in vitro transfection potency of plasmid **DNA-cationic liposome** complexes from 3- to 20-fold. The effect is Ca2+ specific as other cations, such as Mg2+ and Na+, do not give rise to enhanced transfection and the effect can be inhibited by the presence of EGTA. It is shown that Ca2+ increases cellular uptake of the **DNA**-lipid complexes, indicating that increased transfection potency arises from increased intracellular delivery of both cationic lipid and plasmid **DNA** in the presence of Ca2+. In particular, it is shown that the levels of intact intracellular plasmid **DNA** are significantly enhanced when Ca2+ is present. The generality of the Ca2+ effect for enhancing complex-mediated transfection is demonstrated for a number of different cell lines and different cationic lipid formulations. It is concluded that addition of Ca2+ represents a simple and useful protocol for enhancing in vitro transfection properties of plasmid **DNA**-cationic lipid complexes.

ACCESSION NUMBER: 2000:233325 BIOSIS
DOCUMENT NUMBER: PREV200000233325
TITLE: Calcium enhances the transfection potency of plasmid **DNA-cationic liposome** complexes.
AUTHOR(S): Lam, Angela M.I. (1); Cullis, Pieter R.
CORPORATE SOURCE: (1) Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of British Columbia, 2146 Health Sciences Mall, Vancouver, BC, V6T 1Z3 Canada
SOURCE: Biochimica et Biophysica Acta, (Feb. 15, 2000) Vol. 1463, No. 2, pp. 279-290.
ISSN: 0006-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

L5 ANSWER 213 OF 366 BIOSIS COPYRIGHT 2001 BIOSIS
AB In order to identify the important factors involved in **cationic liposome**-mediated gene transfer, in vitro transfection efficiencies by plasmid **DNA** complexed with DOTMA/DOPE liposomes at different **DNA**/liposome mixing ratios were evaluated using four types of cultured cells with respect to their physicochemical properties. Significant changes were observed in the particle size and zeta potential of the complexes as well as in their structures, assessed by atomic force microscopy, which depended on the mixing ratio. In

transfection experiments, except for RAW 264.7 cells (mouse macrophages), efficient gene expression was obtained in MBT-2 cells (mouse bladder tumor), NLH3T3 cells (mouse fibroblasts) and HUVEC (human umbilical vein endothelial cells) at an optimal ratio of 1:5, 1:7.5 or 1:5, respectively.

On the other hand, cellular uptake of the (32P)**DNA**/liposome complexes increased in all cell types with an increase in the mixing ratio, which was not reflected by the transfection efficiency. The cellular damage determined by MTT assay was minimal even at the highest **DNA**/liposome ratio (1:10), indicating that the lower gene expression level at the higher ratio was not due to cytotoxicity induced by the complex. An ethidium bromide intercalation assay showed that the release of plasmid **DNA** from the complex, following the addition of negatively charged liposomes, was restricted as the mixing ratio increased. Furthermore, confocal microscopic studies using HUVEC showed that the 1:5 complexes exhibited a dispersed distribution in the cytoplasm whereas a punctuate intracellular distribution was observed for the 1:10 complexes. This suggests that there was a significant difference in intracellular trafficking, probably release from the endosomes or lysosomes, of the plasmid **DNA**/**cationic liposome** complexes between these mixing ratios. Taken together, these findings suggest that the **DNA**/liposome mixing ratio significantly affects the intracellular trafficking of plasmid **DNA** complexed with the cationic liposomes, which is an important determinant of the optimal mixing ratio in **cationic liposome** -mediated transfection.

ACCESSION NUMBER: 2000:227569 BIOSIS
DOCUMENT NUMBER: PREV200000227569
TITLE: Effect of **DNA**/liposome mixing ratio on the physicochemical characteristics, cellular uptake and intracellular trafficking of plasmid **DNA**/**cationic liposome** complexes and subsequent gene expression.
AUTHOR(S): Sakurai, Fuminori; Inoue, Rui; Nishino, Yasunobu; Okuda, Ayumu; Matsumoto, Osamu; Taga, Tooru; Yamashita, Fumiyoshi; Takakura, Yoshinobu; Hashida, Mitsuru (1)
CORPORATE SOURCE: (1) Department of Drug Delivery Research, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, 606-8501 Japan
SOURCE: Journal of Controlled Release, (May 15, 2000) Vol. 66, No. 2-3, pp. 255-269.
ISSN: 0168-3659.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

L5 ANSWER 214 OF 366 BIOSIS COPYRIGHT 2001 BIOSIS
AB **Cationic liposome-DNA** complexes ('lipoplexes') are used as gene delivery vehicles and may overcome some of the limitations of viral vectors for gene therapy applications. The interaction of highly positively charged lipoplexes with biological macromolecules in blood and tissues is one of the drawbacks of this system. We examined whether coating cationic liposomes with human serum albumin (HSA) could generate complexes that maintained transfection activity. The association of HSA with liposomes composed of 1,2-dioleoyl-3-(trimethylammonium) propane and dioleoylphosphatidyl-ethanolamine, and subsequent complexation with the plasmid pCMVluc greatly increased luciferase expression in epithelial and lymphocytic cell lines above that obtained with plain lipoplexes. The percentage of cells transfected also increased by an order of magnitude. The zeta potential of

the ternary complexes was lower than that of the lipoplexes. Transfection activity by HS[redacted]lipoplexes was not inhibited by[redacted] to 30% serum. The combined use of HSA and a pH-sensitive peptide resulted in significant gene expression in human primary macrophages. HSA-lipoplexes mediated significantly higher gene expression than plain lipoplexes or naked **DNA** in the lungs and spleen of mice. Our results indicate that negatively charged HSA-lipoplexes can facilitate efficient transfection of cultured cells, and that they may overcome some of the problems associated with the use of highly positively charged complexes for gene delivery in vivo.

ACCESSION NUMBER: 2000:227000 BIOSIS
DOCUMENT NUMBER: PREV200000227000
TITLE: Human serum albumin enhances DNA transfection by lipoplexes and confers resistance to inhibition by serum.
AUTHOR(S): Simoes, Sergio; Slepushkin, Vladimir; Pires, Pedro; Gaspar, Rogerio; Pedroso de Lima, Maria C.; Duzgunes, Nejat (1)
CORPORATE SOURCE: (1) Department of Microbiology, School of Dentistry, University of the Pacific, 2155 Webster Street, San Francisco, CA, 94115 USA
SOURCE: Biochimica et Biophysica Acta, (Feb. 15, 2000) Vol. 1463, No. 2, pp. 459-469.
ISSN: 0006-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

L5 ANSWER 215 OF 366 BIOSIS COPYRIGHT 2001 BIOSIS
AB To achieve hepatocyte-targeted in vivo gene expression, a carrier that controls both the tissue and intracellular distribution of DNA was designed and synthesized. A cationic polymer, poly(L-ornithine) (pOrn), was modified first with galactose, then with a fusigenic peptide (mHA2) to obtain Gal-pOrn-mHA2. When applied with Gal-pOrn-mHA2 to asialoglycoprotein receptor-positive cells, fluorescein-labeled DNA showed a diffuse profile, suggesting the release of DNA from endosomes and/or lysosomes by the carrier. Then the biodistribution and gene expression after intravenous injection of DNA complexes (10 mug DNA per mouse) were examined. After injection of (32P)DNA/Gal-pOrn-mHA2, about 60% of the radioactivity was recovered in the liver, mostly in parenchymal cells. A large amount (81 ng/g tissue) of transgene product (luciferase) was detected in the liver of mice injected with DNA/Gal-pOrn-mHA2, which was 280-fold greater than that obtained with DNA/DOTMA:Chol liposomes (50 mug DNA). Prior administration of galactosylated albumin reduced the gene expression to 1/100, indicating the asialoglycoprotein receptor-mediated gene transfer in liver parenchymal cells, ie hepatocytes. The luciferase activity in hepatocytes contributed more than 95% of the total activity in all the tissues examined. Thus, hepatocyte-targeted in vivo gene expression was achieved by the intravenous injection of DNA complex with the multifunctional gene carrier.

ACCESSION NUMBER: 2000:179403 BIOSIS
DOCUMENT NUMBER: PREV200000179403
TITLE: Hepatocyte-targeted in vivo gene expression by intravenous injection of plasmid DNA complexed with synthetic multi-functional gene delivery system.
AUTHOR(S): Nishikawa, M.; Yamauchi, M.; Morimoto, K.; Ishida, E.; Takakura, Y.; Hashida, M. (1)
CORPORATE SOURCE: (1) Department of Drug Delivery Research, Kyoto University, Sakyo-ku, Kyoto, 606-8501 Japan

SOURCE: Gene Therapy, (April, 2000) Vol. 7, No. 7, pp. 548-555.
ISSN: 0969-7128.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

L5 ANSWER 216 OF 366 BIOSIS COPYRIGHT 2001 BIOSIS
AB The present study was aimed at devising an efficient nonviral strategy for suicide gene therapy of hepatocellular carcinoma (HCC). To improve the efficiency of **DNA** delivery and expression, we applied Epstein-Barr virus (EBV)-based plasmid vectors instead of conventional plasmid vectors and combined them with **cationic liposome** (EBV/lipoplex) or polyamidoamine dendrimer (PAAD) (EBV/polyplex). When the beta-galactosidase gene was transferred to HuH7, PLC/PRF/5, or HLE cells, ltoreq50-fold higher beta-galactosidase activities were demonstrated in the cells transfected with EBV vector compared with those transfected with conventional plasmid vectors. PAAD-mediated transfection of HCC with pSES.Tk (an EBV-based vector carrying the herpes simplex virus-1 thymidine kinase gene) resulted in a marked reduction in viable cell number by the addition of ganciclovir (GCV). The HCC cells transfected with pSES.Tk/PAAD showed 100- to 1000-fold higher susceptibilities to GCV than those transfected with pS.Tk (a conventional plasmid vector carrying herpes simplex virus-1 thymidine kinase gene)/PAAD. The pSES.Tk-transfected HCC cells were effectively killed by day 9 in culture with a clinically feasible concentration of GCV (25 muM), whereas the pS.Tk-transfected cells survived the culture. These results demonstrate highly efficient suicide gene transfer into various HCC cells by EBV-based plasmid vectors in vitro, suggesting the possible application of this nonviral vector system to gene therapy of HCC.
ACCESSION NUMBER: 2000:179022 BIOSIS
DOCUMENT NUMBER: PREV200000179022
TITLE: Highly efficient suicide gene expression in hepatocellular carcinoma cells by Epstein-Barr virus-based plasmid vectors combined with polyamidoamine dendrimer.
AUTHOR(S): Harada, Yoshinori; Iwai, Masaki; Tanaka, Saiyu; Okanoue, Takeshi; Kashima, Kei; Maruyama-Tabata, Hiroko; Hirai, Hideyo; Satoh, Etsuko; Imanishi, Jiro; Mazda, Osam (1)
CORPORATE SOURCE: (1) Department of Microbiology, Kyoto Prefectural University of Medicine, Kamikyo, Kyoto, 602-8566 Japan
SOURCE: Cancer Gene Therapy, (Jan., 2000) Vol. 7, No. 1, pp. 27-36.
ISSN: 0929-1903.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

L5 ANSWER 217 OF 366 BIOSIS COPYRIGHT 2001 BIOSIS
AB The skin represents an attractive site for the localised gene therapy of dermatological pathologies and as a potential antigen bioreactor following transdermal delivery. Potential also exists for the gene therapy of skin as a cosmetic intervention. The most exploited non-viral gene delivery system involves the complexation of cationic liposomes with plasmid DNA (pDNA) to form lipid:pDNA vectors that protect the DNA from nuclease-mediated degradation and improve transgene-cell interactions. Despite numerous studies examining the potential for these vectors in delivering genes to a variety of keratinocyte models, investigations into the topical application of such complexes to intact skin tissue is limited. This ex-vivo study, conducted with intact skin tissue derived

from hairless mice, provides quantitative confirmation that topical administration of cationic lipid:pDNA complexes can mediate uptake and expression of reporter pDNA (33-fold higher compared with control) in viable epidermal tissue. The ex-vivo study design provides for intact skin tissue that has not been subjected to depilatory procedures of potential detriment to stratum corneum barrier function, and can be utilised for the quantitative and efficient examination of a potentially wide range of non-viral gene vectors designed for epidermal expression.

ACCESSION NUMBER: 2000:172711 BIOSIS
DOCUMENT NUMBER: PREV200000172711
TITLE: Gene expression in an intact ex-vivo skin tissue model following percutaneous delivery of **cationic liposome**-plasmid **DNA** complexes.
AUTHOR(S): Birchall, James C.; Marichal, Claire; Campbell, Lee; Alwan, Ashraf; Hadgraft, Jonathan; Gumbleton, Mark (1)
CORPORATE SOURCE: (1) Welsh School of Pharmacy, Cardiff University, Cardiff, CF10 3XF UK
SOURCE: International Journal of Pharmaceutics (Kidlington)., (March 20, 2000) Vol. 197, No. 1-2, pp. 233-238. ISSN: 0378-5173.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

L5 ANSWER 218 OF 366 BIOSIS COPYRIGHT 2001 BIOSIS
AB The mechanism of cell entry and intracellular fate of a gene transfer vector composed of a receptor-targeting, **DNA**-condensing peptide, RGD-oligolysine, a luciferase encoding plasmid **DNA** (pDNA) and a **cationic liposome** was examined. We demonstrate by confocal microscopy, electron microscopy and subcellular fractionation that the major mechanism of entry of the vector is endocytic. The vector complex rapidly (5 min) internalizes into early endosomes, then late endosomes and lysosomes. Entry involves, at least in part, clathrin-coated pit-mediated endocytosis since different conditions or drugs known to influence this pathway modify both uptake of pDNA and its expression. The observed increase in expression with addition of a lip some correlated with an increase in the rate of transfer of the pDNA to lysosomes, a decrease in intracellular recycling and exocytosis of the pDNA and an increase in the amount of pDNA in the nuclear fraction. Trafficking within the cell involved endosome fusion and the acid environment of the endosomes-lysosomes was beneficial for expression. After 30 min both the peptide and pDNA localized to the nucleus and the amount of intact pDNA in the nuclear fraction was highest with liposome and peptide. A better understanding of the cellular mechanisms by which vectors transfer to and traffic in cells should help design improved vectors.

ACCESSION NUMBER: 2000:129199 BIOSIS
DOCUMENT NUMBER: PREV200000129199
TITLE: Cell delivery, intracellular trafficking and expression of an integrin-mediated gene transfer vector in tracheal epithelial cells.
AUTHOR(S): Colin, M.; Maurice, M.; Trugnan, G.; Kornprobst, M.; Harbottle, R. P.; Knight, A.; Cooper, R. G.; Miller, A. D.; Capeau, J.; Coutelle, C.; Brahimi-Horn, M. C. (1)
CORPORATE SOURCE: (1) INSERM U 402, Faculte de Medecine Saint-Antoine, 27 rue Chaligny, 75571, Paris Cedex, 12 France
SOURCE: Gene Therapy., (Jan., 2000) Vol. 7, No. 2, pp. 139-152. ISSN: 0969-7128.

DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

L5 ANSWER 219 OF 366 BIOSIS COPYRIGHT 2001 BIOSIS
AB The use of cationic liposomes as nonviral vehicles for the delivery of therapeutic molecules is becoming increasingly prevalent in the field of gene therapy. We have previously demonstrated that the use of the transferrin ligand (Tf) to target a **cationic liposome** delivery system resulted in a significant increase in the transfection efficiency of the complex (Xu, L., Pirollo, K.F., and Chang, E.H. (1997). Hum. Gene Ther. 8, 467-475). Delivery of wild-type (wt) p53 to a radiation-resistant squamous cell carcinoma of the head and neck (SCCHN) cell line via this ligand-targeted, liposome complex was also able to revert the radiation resistant phenotype of these cells in vitro. Here we optimized the Tf/liposome/**DNA** ratio of the complex (LipT) for maximum tumor cell targeting, even in the presence of serum. The efficient reestablishment of wtp53 function in these SCCHN tumor cells in vitro, via the LipT complex, restored the apoptotic pathway, resulting in a significant increase in radiation-induced apoptosis that was directly proportional to the level of exogenous wtp53 in the tumor cells. More significantly, intravenous administration of LipT-p53 markedly sensitized established SCCHN nude mouse xenograft tumors to radiotherapy. The combination of systemic LipT-p53 gene therapy and radiation resulted in complete tumor regression and inhibition of their recurrence even 6 months after the end of all treatment. These results indicate that this tumor-specific, ligand-liposome delivery system for p53 gene therapy, when used in concert with conventional radiotherapy, can provide a new and more effective means of cancer treatment.
ACCESSION NUMBER: 2000:75786 BIOSIS
DOCUMENT NUMBER: PREV200000075786
TITLE: Transferrin-liposome-mediated systemic p53 gene therapy in combination with radiation results in regression of human head and neck cancer xenografts.
AUTHOR(S): Xu, Liang; Pirollo, Kathleen F.; Tang, Wen-Hua; Rait, Antonina; Chang, Esther H. (1)
CORPORATE SOURCE: (1) Lombardi Cancer Center, Georgetown University Medical Center, 3970 Reservoir Road NW, Research Building/E420, Washington, DC USA
SOURCE: Human Gene Therapy, (Dec. 10, 1999) Vol. 10, No. 18, pp. 2941-2952.
ISSN: 1043-0342.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

L5 ANSWER 220 OF 366 BIOSIS COPYRIGHT 2001 BIOSIS
AB In utero injection of **cationic liposome-DNA** complexes (CLDCs) containing chloramphenicol acetyl-transferase, beta-galactosidase (beta-gal), or human granulocyte colony-stimulating factor (hG-CSF) expression plasmids produced high-level gene expression in fetal rats. Tissues adjacent to the injection site exhibited the highest levels of gene expression. Chloramphenicol acetyltransferase expression persisted for at least 14 days and was reexpressed following postnatal reinjection of CLDCs. Intraperitoneal administration of the hG-CSF gene produced high serum hG-CSF levels. X-gal staining demonstrated widespread beta-gal expression in multiple fetal tissues and cell types. No toxic or inflammatory responses were observed, nor was there evidence of fetal-maternal or maternal-fetal gene transfer, suggesting that CLDCs may

provide a useful alternative to viral vectors for in utero gene transfer.
ACCESSION NUMBER: [redacted]00:75564 BIOSIS
DOCUMENT NUMBER: [redacted]REV200000075564
TITLE: Fetal gene transfer by transuterine injection of **cationic liposome-DNA** complexes.
AUTHOR(S): Gaensler, Karin M.L. (1); Tu, Guanhuan; Bruch, Steven; Liggitt, Denny; Lipshutz, Gerald S.; Metkus, Andrea; Harrison, Michael; Heath, Timothy D.; Debs, Robert J.
CORPORATE SOURCE: (1) Department of Medicine, University of California, San Francisco, CA USA
SOURCE: Nature Biotechnology, (Dec., 1999) Vol. 17, No. 12, pp. 1188-1192.
ISSN: 1087-0156.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

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PROCESSING COMPLETED FOR L4
L6 420 DUP REMOVE L4 (148 DUPLICATES REMOVED)

=> d his

(FILE 'HOME' ENTERED AT 14:59:50 ON 19 MAR 2001)

FILE 'CAPLUS, BIOSIS' ENTERED AT 15:00:36 ON 19 MAR 2001
L1 1214 S CATIONIC (A) LIPID
L2 972 S CATIONIC (A) LIPOSOME
L3 972 S CATIONIC(A)LIPOSOME
L4 568 S DNA (S) L1
L5 366 S DNA (S) L2
L6 420 DUP REMOVE L4 (148 DUPLICATES REMOVED)

=> d l6 200 ibib

L6 ANSWER 200 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1999:98047 BIOSIS
DOCUMENT NUMBER: PREV199900098047
TITLE: Novel metabolizable cationic lipids for gene transfer in eukaryotic cells.
AUTHOR(S): Keil, Oliver; Schneider, Manfred P.
CORPORATE SOURCE: FB 9-Bergische, Univ. GH-Wuppertal, D-42097 Wuppertal Germany
SOURCE: Anticancer Research, (Nov.-Dec., 1998) Vol. 18, No. 6C, pp.
4947.
Meeting Info.: Sixth International Conference of Anticancer
Research Kallithea, Halkidiki, Greece October 21-25, 1998
ISSN: 0250-7005.
DOCUMENT TYPE: Conference
LANGUAGE: English

=> d l6 220 ibib

L6 ANSWER 220 OF 420 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:700441 CAPLUS
DOCUMENT NUMBER: 130:34533

TITLE: Colloidal DNA
AUTHOR(S): Podgornik, Rudi; Strey, Hel[redacted] H.; Parsegian, V. Adrian
CORPORATE SOURCE: National Institute of Health, Bethesda, MD, 20892-5626, USA
SOURCE: Curr. Opin. Colloid Interface Sci. (1998), 3(5), 534-539
CODEN: COCSFL; ISSN: 1359-0294
PUBLISHER: Current Chemistry
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
REFERENCE COUNT: 61
REFERENCE(S): (1) Blessing, T; Proc Natl Acad Sci USA 1998, V95, P1427 CAPLUS
(2) Bloomfield, V; Curr Opin Struct Biol 1996, V6, P334 CAPLUS
(3) Bruinsma, R; Eur Phys J B 1998, V4, P75 CAPLUS
(4) Bruinsma, R; Europhys Lett 1998, V41, P165 CAPLUS
(5) Cotten, M; Curr Opin Biotechnol 1993, V4, P705 CAPLUS
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=> d l6 230 ibib

L6 ANSWER 230 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 81
ACCESSION NUMBER: 1998:122424 CAPLUS
DOCUMENT NUMBER: 128:254865
TITLE: Analytical methods for the characterization of cationic lipid-nucleic acid complexes
AUTHOR(S): Ferrari, Marilyn E.; Nguyen, Cuong M.; Zelphati, Olivier; Tsai, Yali; Felgner, Philip L.
CORPORATE SOURCE: Vical Inc., San Diego, CA, 92121, USA
SOURCE: Hum. Gene Ther. (1998), 9(3), 341-351
CODEN: HGTHE3; ISSN: 1043-0342
PUBLISHER: Mary Ann Liebert, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

=> d l6 240 ibib

L6 ANSWER 240 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 87
ACCESSION NUMBER: 1999:168319 CAPLUS
Correction of: 1998:9222
DOCUMENT NUMBER: 130:163663
Correction of: 128:84876
TITLE: Synthesis, Activity, and Structure-Activity Relationship Studies of Novel Cationic Lipids for DNA Transfer
AUTHOR(S): Byk, Gerardo; Dubertret, Catherine; Escriou, Virginie;
Frederic, Marc; Jaslin, Gabrielle; Rangara, Ravi; Pitard, Bruno; Crouzet, Joel; Wils, Pierre; Schwartz, Bertrand; Scherman, Daniel
CORPORATE SOURCE: Gencell/Rhone-Poulenc Rorer, Vitry sur Seine, 94403, Fr.
SOURCE: J. Med. Chem. (1998), 41(2), 224-235
CODEN: JMCMAR; ISSN: 0022-2623
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

=> d l6 250 ibib

L6 ANSWER 250 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 94
ACCESSION NUMBER: 1998:264750 CAPLUS
DOCUMENT NUMBER: 129:104860
TITLE: Reproducible and efficient murine CNS gene delivery using a microprocessor-controlled injector
AUTHOR(S): Brooks, Andrew I.; Halterman, Marc W.; Chadwick, Christopher A.; Davidson, Beverly L.; Haak-Frendscho, Mary; Radel, Clyde; Porter, Chris; Federoff, Howard J.
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY, 14642, USA
SOURCE: J. Neurosci. Methods (1998), 80(2), 137-147
CODEN: JNMEDT; ISSN: 0165-0270
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

=> d l6 260 ibib

L6 ANSWER 260 OF 420 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:116859 CAPLUS
DOCUMENT NUMBER: 128:188898
TITLE: **Cationic lipid** effects on **DNA** conformation and on genetic transformation
AUTHOR(S): Yoshikawa, Yuko; Emi, Nobuhiko; Yoshikawa, Kenichi
CORPORATE SOURCE: Nagoya Bunri Jr. Coll., Japan
SOURCE: Kagaku (Kyoto) (1998), 53(2), 70-71
CODEN: KAKYAU; ISSN: 0451-1964
PUBLISHER: Kagaku Dojin
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese

=> d l6 300 ibib

L6 ANSWER 300 OF 420 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:166340 CAPLUS
DOCUMENT NUMBER: 126:268433
TITLE: Optimization of formulations and conditions for the aerosol delivery of functional **cationic lipid:DNA** complexes
AUTHOR(S): Eastman, Simon J.; Tousignant, Jennifer D.; Lukason, Michael J.; Murray, Heather; Siegel, Craig S.; Constantino, Paul; Harris, David J.; Cheng, Seng H.; Scheule, Ronald K.
CORPORATE SOURCE: Genzyme Corporation, Framingham, MA, 0701-9322, USA
SOURCE: Hum. Gene Ther. (1997), 8(3), 313-322
CODEN: HGTHE3; ISSN: 1043-0342
PUBLISHER: Liebert
DOCUMENT TYPE: Journal
LANGUAGE: English

=> d l6 280 ibib

L6 ANSWER 280 OF 420 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:770778 CAPLUS
DOCUMENT NUMBER: 128:97267
TITLE: Development and characterization of cationic liposomes conjugated with HVJ (Sendai virus): reciprocal effect of cationic lipid for in vitro and in vivo gene transfer
AUTHOR(S): Saeki, Yoshinaga; Matsumoto, Norinao; Nakano, Yoshiro; Mori, Masato; Awai, Koji; Kaneda, Yasufumi
CORPORATE SOURCE: Institute for Molecular and Cellular Biology, Osaka University, Suita, 565, Japan
SOURCE: Hum. Gene Ther. (1997), 8(17), 2133-2141
CODEN: HGTHE3; ISSN: 1043-0342
PUBLISHER: Mary Ann Liebert, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

=> d l6 270 ibib

L6 ANSWER 270 OF 420 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:568307 CAPLUS
DOCUMENT NUMBER: 127:230340
TITLE: Complexes of DNA, cationic lipids and membrane-active peptides for introduction of DNA into higher eukaryotic cells
INVENTOR(S): Wagner, Ernst; Mechtler, Karl; Kichler, Antoine
PATENT ASSIGNEE(S): Boehringer Ingelheim International G.m.b.H., Germany; Wagner, Ernst; Mechtler, Karl; Kichler, Antoine
SOURCE: PCT Int. Appl., 62 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9730170	A1	19970821	WO 1997-EP649	19970213
W: CA, JP, MX, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
DE 19605548	A1	19970904	DE 1996-19605548	19960215
CA 2246227	AA	19970821	CA 1997-2246227	19970213
EP 900281	A1	19990310	EP 1997-904426	19970213
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000504579	T2	20000418	JP 1997-528984	19970213
PRIORITY APPLN. INFO.:			DE 1996-19605548	19960215
			WO 1997-EP649	19970213

=> d l6 260-270 abs ibib

L6 ANSWER 260 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB A review with 10 refs. on effects of cationic lipids on DNA conformation, and enhancement of DNA transformation into cells by cationic lipids (e.g., N-2,3-(dioleyloxy)-propyl-N,N,N-trimethylammonium chloride (DOTMA)) and by cationic polymers.
ACCESSION NUMBER: 1998:116859 CAPLUS

DOCUMENT NUMBER: 128:188898
TITLE: **Cationic lipid** effects on **DNA** conformation and on genetic transformation
AUTHOR(S): Yoshikawa, Yuko; Emi, Nobuhiko; Yoshikawa, Kenichi
CORPORATE SOURCE: Nagoya Bunri Jr. Coll., Japan
SOURCE: Kagaku (Kyoto) (1998), 53(2), 70-71
CODEN: KAKYAU; ISSN: 0451-1964
PUBLISHER: Kagaku Dojin
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese

L6 ANSWER 261 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB A review with 24 refs. At. force microscopy studies of DNA attached to rigid surfaces were initially motivated by the development of methods which would stretch DNA for the purposes of rapid sequencing. Currently there is much interest in studies of multilayers of DNA chains self-assembled on membranes which form spontaneously when DNA adsorbs onto oppositely charged cationic liposomes (CLs). A major motivation for elucidating the structures and interactions in these CL-DNA complexes arises for two reasons. The first is that they are known to mimic certain characteristics of viruses by being efficient chem. carriers of genes (DNA sections) for delivery in cells. The second is that they are models of studies of DNA condensation phases in two dimensions. DNA-membrane interactions should also provide clues for the relevant mol. forces in the condensation of DNA in chromosomes and viral capsids. The particular complexes described in this review contain linear DNA forming a new "hybrid" phase of matter; i.e., the DNA chains form a finite size two dimensional smectic coupled to the three dimensional smectic phase of membranes.
ACCESSION NUMBER: 1998:174284 CAPLUS
DOCUMENT NUMBER: 128:305169
TITLE: DNA at membrane surfaces: an experimental overview
AUTHOR(S): Safinya, Cyrus R.; Koltover, Ilya; Raedler, Joachim
CORPORATE SOURCE: Materials and Physics Departments, Biochemistry and Molecular Biology Program, University of California, Santa Barbara, CA, 93106, USA
SOURCE: Curr. Opin. Colloid Interface Sci. (1998), 3(1), 69-77
CODEN: COCSFL; ISSN: 1359-0294
PUBLISHER: Current Chemistry
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

L6 ANSWER 262 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB **Cationic lipid** formulations for gene transfer usually consist of a **cationic lipid**, a neutral lipid and the plasmid **DNA** of interest. Each of these components must be optimized in order for the highest level of gene transfer to be achieved. Detailed anal. of cationic lipid structure activity relationships have revealed a no. of structural components that are important for high gene transfer efficiency. The examn. of a no. of neutral lipids as alternatives to the commonly used DOPE resulted in the identification of formulations that had enhanced activity with diphantanoylPE as the neutral lipid. An important feature for optimization in the plasmid component of the formulation is persistent expression. Co-installation of a plasmid contg. the adenovirus E4 region with a reporter gene plasmid gave a significant increase in persistence.
ACCESSION NUMBER: 1998:524711 CAPLUS
TITLE: Structure activity relationships in the development of

cationic lipids for efficient gene transfer to the lung.

AUTHOR(S): Harris, D. J.; Marshall, J.; Lee, E. R.; Siegel, C. S.; Yew, N. S.; Nietupski, J. B.; Rafter, P. W.; Lane, M. B.; Rudginsky, S. A.; Armentano, D.; Cheng, S. H.

CORPORATE SOURCE: Genzyme Corporation, Cambridge, MA, 02139-1562, USA

SOURCE: Book of Abstracts, 216th ACS National Meeting, Boston, August 23-27 (1998), BIOT-052. American Chemical Society: Washington, D. C.
CODEN: 66KYA2

DOCUMENT TYPE: Conference; Meeting Abstract

LANGUAGE: English

L6 ANSWER 263 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 99

AB Previously, we have described the optimization of the aerosol delivery of a nonviral gene therapy vector to the lungs of rodents (Eastman et al., 1997). Although aerosolizing cationic lipid:pDNA complexes into a whole-body exposure chamber resulted in high levels of reporter gene expression in the lungs of BALB/c mice, the conditions employed were not optimal for the delivery of lipid:pDNA complexes to the lungs of human patients. I.e., the consumption rate of the material in the nebulizer, and thus the delivery time, were very slow and the aerosol was delivered in a continuous flow. Here we describe in vitro expts. used to develop a cationic lipid:pDNA aerosol with characteristics more suitable for delivery to the lungs of humans, as a necessary prerequisite for conducting a clin. study with human cystic fibrosis patients. Using cascade impactors and all-glass impingers, we have screened several com. available nebulizers for their ability to deliver intact, respirable, active lipid:pDNA complexes in the shortest time possible, and have identified the Pari LC Jet Plus nebulizer as the optimal nebulizer that meets these criteria. Using this nebulizer in an intermittent mode to mimic breath actuation, consumption rates of approx. 0.6 mL/min of the cationic lipid:pDNA complexes (6 mM cationic lipid:8 mM pDNA) were obtained. The plasmid DNA remained intact and the complexes were shown to maintain activity throughout the nebulization run. Based on measurements of the nebulized dose and the mass median aerodynamic diam., we calc. a delivered dose of .apprx.22 .mu.mol (7.2 mg) of pDNA for each 8 mL of cationic lipid:pDNA complex aerosolized to the lungs of a human patient. This dose should be sufficient to test the clin. efficacy of cationic lipid-mediated gene delivery for the treatment of cystic fibrosis.

ACCESSION NUMBER: 1998:54894 CAPLUS

DOCUMENT NUMBER: 128:208840

TITLE: Aerosolization of cationic lipid:pDNA complexes-in vitro optimization of nebulizer parameters for human clinical studies

AUTHOR(S): Eastman, Simon J.; Tousignant, Jennifer D.; Lukason, Michael J.; Chu, Qiuming; Cheng, Seng H.; Scheule, Ronald K.

CORPORATE SOURCE: Genzyme Corporation, Framingham, MA, 01701-9322, USA

SOURCE: Hum. Gene Ther. (1998), 9(1), 43-52
CODEN: HGTHE3; ISSN: 1043-0342

PUBLISHER: Mary Ann Liebert, Inc.

DOCUMENT TYPE: Journal

LANGUAGE: English

L6 ANSWER 264 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

AB A new series of gene carriers, polyethylene glycol (PEG)-grafted poly-L-lysine (PLL, mol. wt. =25 000) with three different PEG-grafted ratios (5, 10 and 25 mole%, which means 5, 10 and 25% of epsilon-amino group of PLL was modified by PEG), was synthesized. These new gene carriers, named comb-shaped PEG-g-PLL copolymer, showed a 5- to 30-fold increase in transfection efficiency compared to PLL alone on a human

carcinoma cell line. it is likely that Hep G2 cells were transfected by plasmid **DNA**/PE[redacted]-PLL complexes through an endo[redacted]osis mechanism due to the fact that chloroquine increased trans[redacted]ection efficiency. Although Lipofectin, a **cationic lipid** formulation, showed slightly higher transfection efficiency than PEG-g-PLL in Hep G2 cells, our designed PEG-g-PLL demonstrated lower cytotoxicity, early gene expression and maintenance of gene expression for up to 96 h.

ACCESSION NUMBER: 1998:302637 BIOSIS
DOCUMENT NUMBER: PREV199800302637
TITLE: Polyethylene glycol-grafted poly-L-lysine as polymeric gene carrier.
AUTHOR(S): Choi, Young Hun; Liu, Feng; Kim, Jin-Seok; Choi, Young Kweon; Park, Jong Sang; Kim, Sung Wan (1)
CORPORATE SOURCE: (1) Center Controlled Chem. Delivery, Dep. Pharmaceutics Pharmaceutical Chem., Univ. Utah, 570 Biomedical Polymers Res. Build., Room 205, Salt Lake City, UT 84112 USA
SOURCE: Journal of Controlled Release, (June, 1998) Vol. 54, No. 1, pp. 39-48.
ISSN: 0168-3659.
DOCUMENT TYPE: Article
LANGUAGE: English

L6 ANSWER 265 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 100
AB The aim of this study is to get a better understanding of **DNA**-**cationic lipid** complex formation and its characterization through the properties of the lipid assembly, using fluorescent probes known to have different locations in the vesicle bilayer, 1,6-diphenylhexa-1,3,5-triene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMADPH). The location of these two fluorescent probes in the membrane differs; the pos. charge of TMADPH is localized close to the water/lipid interface and its fluorophore is present in the upper part of the acyl chain region while DPH (lacking polar group) is embedded deeper in the hydrophobic part of the bilayer. Unilamellar vesicles (.apprx.100 nm size) composed of N-(1-(2,3-dioleoyloxy)-propyl)-N,N,N-trimethylammonium chloride (DOTAP) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) as a helper lipid (at 1:1 mol ratio) were used as a model of cationic liposomes. Both linear and circular DNA gave almost identical results. DNA-/L+ (mole charge ratio of DNA neg.-charged phosphate to pos.-charged lipid) ratios have large effects on the measured parameters. The effects monitored through TMADPH are much more striking than those obtained through the use of DPH, suggesting that the major DNA-lipid interaction occurs at the lipid/water interface. The fact that DNA induced much larger changes in TMADPH fluorescence intensity in H2O than in D2O suggests that the changes in the exposure of TMADPH to water and solvent relaxation effects are involved in the interaction. At DNA-/L+.gtoreq.1, fluorescence intensity increased concomitantly with a small increase in TMADPH fluorescence anisotropy without much affect in the size of the complex. At DNA-/L+<0.6, fluorescence quenching proportional to DNA-/L+ occurred, as well as a large increase in TMADPH fluorescence anisotropy and in complex size. These results suggest that at low DNA-/L+, neg.-charged DNA condenses pos.-charged lipid headgroups, thereby inducing formation of lipid-ordered domains. This phase sepn. results in membrane defects at the lipid/water interface and increased exposure of the hydrophobic upper parts of the acyl chains to water, as indicated by the quenching of TMADPH. This leads to instability and aggregation/fusion of the DNA-lipid complexes. On the other hand, at DNA-/L+.gtoreq.1, the condensing effect is smaller, involving homogeneous lateral condensation of all the lipids, leading to a redn. in water content near the probe, and the DNA-lipid

complexes are relatively small and stable.
ACCESSION NUMBER: 1998:152911 CAPLUS
DOCUMENT NUMBER: 128:305242
TITLE: Probing **DNA-cationic lipid** interactions with the fluorophore trimethylammonium diphenyl-hexatriene (TMADPH)
AUTHOR(S): Hirsch-Lerner, Danielle; Barenholz, Yechezkel
CORPORATE SOURCE: P.O. Box 12272, Hadassah Medical School, Department of Biochemistry, The Hebrew University, Jerusalem, 91120, Israel
SOURCE: Biochim. Biophys. Acta (1998), 1370(1), 17-30
CODEN: BBACAQ; ISSN: 0006-3002
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

L6 ANSWER 266 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB We study fluctuations of **DNA-cationic lipid** complexes in their lamellar membrane phases with **DNA** intercalated between lipid membranes. We theor. elucidate this novel state of matter by characterizing it as the very first realization of a decoupled (unregistered) phase of strongly fluctuating 2-d smectic manifolds weakly interacting across membranes. Due to couplings between adjacent 2-d smectic Lx .times. Ly planes, the exptl. obsd. ordinary 2-d smectic behavior [Salditt et al., Phys. Rev. Lett. 79, 2582 (1997)] of DNA in-plane undulations, with < u2 > .apprx. Ly1/2 .apprx. Lx, must cross over, at the longest scales, to a novel fluctuation behavior, with < u2 > .apprx. (log Lg)2 - (log Lx)2.
ACCESSION NUMBER: 1998:782570 CAPLUS
DOCUMENT NUMBER: 130:106682
TITLE: Quasi-two-dimensional smectic states of DNA molecules intercalated between lipid membranes in multi-lamellar phases
AUTHOR(S): Golubovic, Leonardo; Moldovan, Dorel; Golubovic, Mirjana
CORPORATE SOURCE: Physics Department, West Virginia University, Morgantown, WV, 26506, USA
SOURCE: Mater. Res. Soc. Symp. Proc. (1998), 489(Materials Science of the Cell), 13-18
CODEN: MRSPDH; ISSN: 0272-9172
PUBLISHER: Materials Research Society
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 10
REFERENCE(S): (2) Golubovic, L; Phys Rev A 1991, V43, P6793 CAPLUS
(3) Golubovic, L; Phys Rev E 1994, V49, P2567 CAPLUS
(4) Golubovic, L; Phys Rev Lett 1990, V65, P1963 CAPLUS
(6) Kamien, R; Phys Rev Lett 1995, V74, P2499 CAPLUS
(8) Salditt, T; Phys Rev Lett 1997, V79, P2582 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 267 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1998:355992 BIOSIS
DOCUMENT NUMBER: PREV199800355992
TITLE: Static headspace gas chromatographic analysis of the residual solvents in cationic lipid-based gene transfer agents.
AUTHOR(S): Chang, Chau-Dung (1); Boguslavskaya, Marina
CORPORATE SOURCE: (1) Dep. Chem., Genzyme Corp., Cambridge, MA 02139 USA
SOURCE: American Biotechnology Laboratory, (July, 1998) Vol. 16,

No. 8, pp. 11-12.
SSN: 0749-3223.
DOCUMENT TYPE: rticle
LANGUAGE: English

L6 ANSWER 268 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 101
AB Cationic liposomes retain a significant trapped vol. after their complexation to plasmid DNA. This is significant for understanding the phys. nature of liposome/plasmid DNA complexes used in gene therapy and the potential for codelivery of other encapsulated mols. with the liposome-DNA complexes. Cationic liposomes composed of N,N-dioleoyl-N,N-dimethylammonium chloride and dioleoylphosphatidylethanolamine (DODAC/DOPE, 50/50 mol%) encapsulating an aq. trap marker were used to prep. liposome-DNA complexes at various charge ratios. The trapped vol. before and after DNA binding was measured by dialysis and by filtration. The effect of tissue culture medium on trapped vol. was also investigated. A lipid-mixing assay was employed to further characterize the aggregation events that influence trap vol. The trapped vol. (Vt) of neutral control liposomes was 1.1 .mu.L/.mu.mol, which was not affected by the addn. of DNA. For cationic liposomes in the absence of DNA, the Vt was 1.45 and 1.54 .mu.L/.mu.mol as measured by the filtration and dialysis methods, resp. After addn. of DNA, the residual trapped vol. (RVt) decreased to 0.43 and 0.47 .mu.L/.mu.mol, as detd. by each method, resp. RVt increased as the ratio of **cationic lipid** to **DNA** (nmol lipid/mg **DNA**) increased above 10, a ratio that corresponds to a charge ratio (pos. charged lipids to neg. charged phosphate groups) of 1.62. Aggregation and lipid mixing were greatest at charge ratios coinciding with the lowest trapped vol. In the presence of tissue culture medium, the Vt of cationic liposomes (but not neutral liposomes) was reduced, suggesting that the salts have a direct effect on cationic liposomes in the absence of DNA. The RVt of both neutral and cationic liposomes in the presence of DNA, however, was not different from that of the liposomes in the absence of DNA. That a significant trapped vol. is retained by cationic liposomes after binding to plasmid DNA is important with regard to the potential use of DNA/liposome complexes in the codelivery of other bioactive mols. at the time of cell transfection.
ACCESSION NUMBER: 1997:786666 CAPLUS
DOCUMENT NUMBER: 128:16385
TITLE: Cationic liposome-plasmid DNA complexes used for gene transfer retain a significant trapped volume
AUTHOR(S): Wasan, Ellen K.; Fairchild, Alysa; Bally, Marcel B.
CORPORATE SOURCE: Department of Advanced Therapeutics B. C. Cancer Agency and the Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Can.
SOURCE: J. Pharm. Sci. (1998), 87(1), 9-14
CODEN: JPMSAE; ISSN: 0022-3549
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

L6 ANSWER 269 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB Cationic lipids R1R2(R3)iN+(CH2)mXp(CH2)nN+R1'R2'(R3')i 2Y- [X = O, S(O), CH2; Y = anion; R1, R1' = C1-18 aliph.; R2, R2' = H, C1-18 alkyl, cyanoethyl, aminopropyl, aminobutyl, C2-4 alkyl guanidinium or amidinium, etc.; R3, R3' = C1-6 alkyl, AcOCH2CH2, CH2CO2Et; m, n = 1-3; i, p = 0, 1] can be used alone or in mixts. with other liposome-forming compds. to prep. lipid aggregates to serve as carriers for transfection of nucleic acids or delivery of other neg. charged macromols. into animal cells and are therefore useful in gene therapy. Some of these lipids are also

useful as detergents for cleaning and as vehicles in cosmetics. Thus, a mixt. of .beta[redacted]alactosidase DNA and N,N,N',N'[redacted] guanidinopropyl[redacted]ctadecyloxybis-2,2'-ethylamine-HI (prepn. given) was incubated with primary human epidermal keratinocytes for 4 h. After medium replacement and addnl. incubation for 48 h, 50% of the cells tested pos. for .beta.-galactosidase.

ACCESSION NUMBER: 1997:752808 CAPLUS
DOCUMENT NUMBER: 128:53227
TITLE: Cationic lipids for transfection of negatively charged or neutral molecules into living cells
INVENTOR(S): Haces, Alberto
PATENT ASSIGNEE(S): Haces, Alberto, USA
SOURCE: PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9742819	A1	19971120	WO 1997-US9093	19970509

W: CA, IL, JP, US
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

PRIORITY APPLN. INFO.: US 1996-17298 19960513
OTHER SOURCE(S): MARPAT 128:53227

L6 ANSWER 270 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB A carrier system for the introduction of transforming **DNA** into higher eukaryotic cells complexes the nucleic with a **cationic lipid** present in a suboptimal concn. for transfection and one or more membrane-active acidic peptides and optionally helper lipid. The ratio of the total no. of pos. charges to the total no. of neg. charges in the compn. is between approx. 0 and approx. 3. Optimization expts. are reported. One finding was that the use of acidic peptides in the complex lessened the inhibiting effect of serum on transformation. High charge ratios also made the uptake independent of vacuolar proton exchange.

ACCESSION NUMBER: 1997:568307 CAPLUS
DOCUMENT NUMBER: 127:230340
TITLE: Complexes of DNA, cationic lipids and membrane-active peptides for introduction of DNA into higher eukaryotic cells
INVENTOR(S): Wagner, Ernst; Mechtler, Karl; Kichler, Antoine
PATENT ASSIGNEE(S): Boehringer Ingelheim International G.m.b.H., Germany; Wagner, Ernst; Mechtler, Karl; Kichler, Antoine
SOURCE: PCT Int. Appl., 62 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9730170	A1	19970821	WO 1997-EP649	19970213

W: CA, JP, MX, US
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

DE 19605548	A1	19970904	DE 1996-19605548	19960215
CA 2246227	AA	19970821	CA 1997-2246227	19970213
EP 900281	A1	19990310	EP 1997-904426	19970213

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

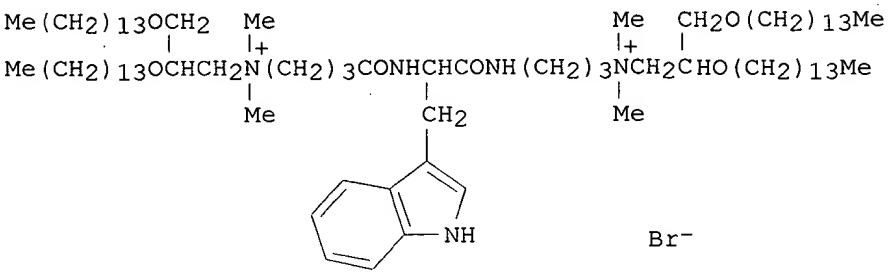
JP 2000504579 T2 20000418 JP 1997-528984 19970213
PRIORITY APPLN. INFO.: DE 1996-19605548 19960215
WO 1997-EP649 19970213

=> s (dimer? or oligomer?) (s) l6

L7 1 (DIMER? OR OLIGOMER?) (S) L6

=> d l7 abs ibib

L7 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS
GI



I

AB A compn. is provided comprising a novel cationic lipid compd. having hydrophobic tails and two quaternary ammonium headgroups bridged by a linker. The compn. is useful as a cytofectin for facilitating delivery and transfection of biol. active agents, particularly anionic bioactive agents such as DNA, into cells. The compn. is useful also as an adjuvant for enhancing the humoral immune response of a vertebrate to an immunogen, esp. an immunogen encoded by a polynucleotide-based vaccine. In certain preferred embodiments, the **cationic lipid** compd. is a **dimer** contg. quaternary ammonium headgroups bridged by a linker having **DNA** and/or cell receptor binding affinity, such as a polypeptide or polyamine. Also disclosed is an immunogenic compn. comprising an immunogen and the compn. of the present invention. I was prepd. as an example compd.

ACCESSION NUMBER: 2000:861646 CAPLUS
DOCUMENT NUMBER: 134:21482
TITLE: Cytofectin dimers and methods of use thereof
INVENTOR(S): Wheeler, Carl J.
PATENT ASSIGNEE(S): Vical, Inc., USA
SOURCE: PCT Int. Appl., 50 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000073263	A1	20001207	WO 2000-US14676	20000526

W: CA, JP, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

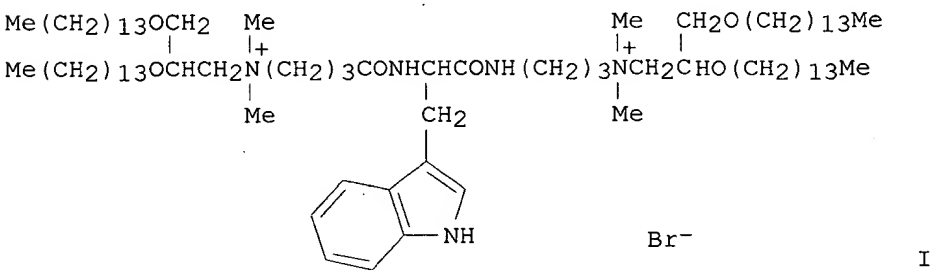
PRIORITY APPLN. INFO.: US 1999-136472 19990528
OTHER SOURCE(S): MARPAT 134:21482
REFERENCE COUNT: 5
REFERENCE(S):
(1) Carl, J; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES 1996, V93, P11454
(2) Philip, L; US 5459127 A 1995 CAPLUS
(3) Santanu, B; JOURNAL OF THE CHEMICAL SOCIETY, CHEMICAL COMMUNICATIONS 1997, 23, P2287
(4) The Regents Of The University Of California; WO 9711935 A 1997 CAPLUS
(5) Vical; WO 9719675 A 1997 CAPLUS

=> s (link? or bind?) (s) l6

L8 27 (LINK? OR BIND?) (S) L6

=> d l7 1-10 abs ibib

L7 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS
GI



I

AB A compn. is provided comprising a novel cationic lipid compd. having hydrophobic tails and two quaternary ammonium headgroups bridged by a linker. The compn. is useful as a cytofectin for facilitating delivery and transfection of biol. active agents, particularly anionic bioactive agents such as DNA, into cells. The compn. is useful also as an adjuvant for enhancing the humoral immune response of a vertebrate to an immunogen, esp. an immunogen encoded by a polynucleotide-based vaccine. In certain preferred embodiments, the **cationic lipid** compd. is a **dimer** contg. quaternary ammonium headgroups bridged by a linker having **DNA** and/or cell receptor binding affinity, such as a polypeptide or polyamine. Also disclosed is an immunogenic compn. comprising an immunogen and the compn. of the present invention. I was prepd. as an example compd.

ACCESSION NUMBER: 2000:861646 CAPLUS
DOCUMENT NUMBER: 134:21482
TITLE: Cytofectin dimers and methods of use thereof
INVENTOR(S): Wheeler, Carl J.
PATENT ASSIGNEE(S): Vical, Inc., USA
SOURCE: PCT Int. Appl., 50 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000073263	A1	20001207	WO 2000-US14676	20000526

W: CA, JP, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

PRIORITY APPLN. INFO.: US 1999-136472 19990528
OTHER SOURCE(S): MARPAT 134:21482
REFERENCE COUNT: 5
REFERENCE(S):
(1) Carl, J; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES 1996, V93, P11454
(2) Philip, L; US 5459127 A 1995 CAPLUS
(3) Santanu, B; JOURNAL OF THE CHEMICAL SOCIETY, CHEMICAL COMMUNICATIONS 1997, 23, P2287
(4) The Regents Of The University Of California; WO 9711935 A 1997 CAPLUS
(5) Vical; WO 9719675 A 1997 CAPLUS

=> d l7 1-27 py

L7 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS
PY 2000

=> d l7 2-10 abs ibib

1 ANSWERS ARE AVAILABLE. SPECIFIED ANSWER NUMBER EXCEEDS ANSWER SET SIZE
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NUMBERS IN RANGE MUST BE GREATER THAN ZERO.
An answer number must be a positive number.
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L7 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS
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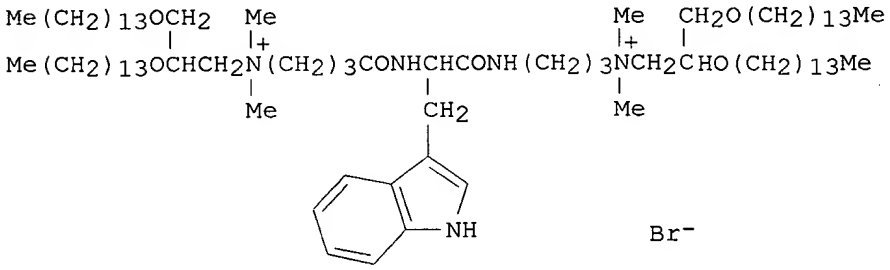

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esp. an immunogen encoded by a polynucleotide-based vaccine. In certain preferred embodiments, the **cationic lipid** compounds a **dimer** contg. quaternary ammonium headgroups bridged by a linker having **DNA** and/or cell receptor binding affinity, such as a polypeptide or polyamine. Also disclosed is an immunogenic compn. comprising an immunogen and the compn. of the present invention. I was prepd. as an example compd.

ACCESSION NUMBER: 2000:861646 CAPLUS
DOCUMENT NUMBER: 134:21482
TITLE: Cytofectin dimers and methods of use thereof
INVENTOR(S): Wheeler, Carl J.
PATENT ASSIGNEE(S): Vical, Inc., USA
SOURCE: PCT Int. Appl., 50 pp.
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DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000073263	A1	20001207	WO 2000-US14676	20000526

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RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

PRIORITY APPLN. INFO.: US 1999-136472 19990528
OTHER SOURCE(S): MARPAT 134:21482
REFERENCE COUNT: 5
REFERENCE(S):
(1) Carl, J; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES 1996, V93, P11454
(2) Philip, L; US 5459127 A 1995 CAPLUS
(3) Santanu, B; JOURNAL OF THE CHEMICAL SOCIETY, CHEMICAL COMMUNICATIONS 1997, 23, P2287
(4) The Regents Of The University Of California; WO 9711935 A 1997 CAPLUS
(5) Vical; WO 9719675 A 1997 CAPLUS

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(FILE 'HOME' ENTERED AT 14:59:50 ON 19 MAR 2001)

FILE 'CAPLUS, BIOSIS' ENTERED AT 15:00:36 ON 19 MAR 2001
L1 1214 S CATIONIC (A) LIPID
L2 972 S CATIONIC (A) LIPOSOME
L3 972 S CATIONIC(A)LIPOSOME
L4 568 S DNA (S) L1
L5 366 S DNA (S) L2
L6 420 DUP REMOVE L4 (148 DUPLICATES REMOVED)
L7 1 S (DIMER? OR OLIGOMER?) (S) L6
L8 27 S (LINK? OR BIND?) (S) L6

=> d l8 1-27 py

L8 ANSWER 1 OF 27 CAPLUS COPYRIGHT 2001 ACS
PY 2000

L8 ANSWER 2 OF 27 CAPLUS COPYRIGHT 2001 ACS
PY 2000

L8 ANSWER 3 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 4 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 5 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 6 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 7 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 8 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 9 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 10 OF 27 CAPLUS COPYRIGHT 2001 ACS
PY 1997

L8 ANSWER 11 OF 27 CAPLUS COPYRIGHT 2001 ACS
PY 1996
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L8 ANSWER 12 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 13 OF 27 CAPLUS COPYRIGHT 2001 ACS
PY 1996

L8 ANSWER 14 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 15 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 16 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 17 OF 27 CAPLUS COPYRIGHT 2001 ACS
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L8 ANSWER 18 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
PY 2000

L8 ANSWER 19 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
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L8 ANSWER 20 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
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L8 ANSWER 21 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
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L8 ANSWER 22 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS

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L8 ANSWER 23 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
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L8 ANSWER 24 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
PY 1997

L8 ANSWER 25 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
PY 1996

L8 ANSWER 26 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
PY 1995

L8 ANSWER 27 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
PY 1995

=> d l8 24-27 abs ibib

L8 ANSWER 24 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
AB Purpose. We have demonstrated that a heteromolecular complex consisting of cationic lipids and **DNA** can be prepared and isolated (I). Cationic lipids **bind DNA** through electrostatic interactions. However, when sufficient lipids are bound to **DNA** the physical and chemical properties of the complex are governed by hydrophobic effects. Here we describe an approach where this hydrophobic complex is used as an intermediate in the preparation of lipid-**DNA** particles (LDPs). Methods. The approach relies on the generation of mixed micelles containing the detergent, n-octyl beta-D-glucopyranoside (OGP), the **cationic lipid**, N-N-dioleoyl-N, N-dimethylammonium chloride (DODAC), and selected zwitterionic lipids, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) or egg sphingomyelin (SM). Results. When these micelles were prepared at low detergent concentrations (20 mM OGP) and combined with pCMV-beta **DNA**, LDPs spontaneously formed. The mean diameter of these particles as measured by quasielastic light scattering was 55-70 nm, a result that was confined by negative stain electron microscopy. Further characterization of these LDPs showed that **DNA** within the particles was inaccessible to the small fluorochrome TO-PRO-1 and protected against DNase I degradation. LDPs could also be prepared in high concentrations of OGP (100 mM), however particles formed only after removal of OGP by dialysis. Particles formed in this manner were large (gt 2000nm) and mediated efficient transfection of Chinese hamster ovary cells. Transfection activity was greater when the lipid composition used consisted of SM/DODAC. Small particles (lt 100nm) prepared of SM/DODAC were, however, inefficient transfecting agents. Conclusions. We believe that LDP formation is a consequence of the molecular forces that promote optimal hydrocarbon-hydrocarbon interactions and elimination of the hydrocarbon-water interface.
ACCESSION NUMBER: 1997:203487 BIOSIS
DOCUMENT NUMBER: PREV199799502690
TITLE: Self-assembling DNA-lipid particles for gene transfer.
AUTHOR(S): Zhang, Yuan-Peng; Reimer, Dorothy L.; Zhang, Guoyang; Lee, Patricia H.; Bally, Marcel B. (1)
CORPORATE SOURCE: (1) British Columbia Cancer Agency, Div. Med. Oncology, Sect. Advanced Therapeutics, 600 W. 10th Ave., Vancouver, BC V5Z 4E6 Canada
SOURCE: Pharmaceutical Research (New York), (1997) Vol. 14, No. 2, pp. 190-196.
ISSN: 0724-8741.

DOCUMENT TYPE: Article
LANGUAGE: English

L8 ANSWER 25 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
AB Cationic amphiphiles have been shown to mediate gene transfer to eukaryotic cells, although the nature and fate of the lipid-**DNA** complexes is still a matter of debate. Negative staining transmission electron microscopy (TEM) of the complexes in physiological medium, as well as thin-section TEM of transfected cells has been used to visualize the particles and the possible pathways leading to transgene expression. Lipopolyamines form a network of tubular micelles into which plasmid **DNA** is intertwined and condensed; the cationic particles contain hundreds of plasmid molecules and are heterogeneous with respect to size (0.1-0.5 mu) and shape. Adherent cells (293M, 3T3, MRC5, primary leptomeningeal cells) take them up readily within minutes by spontaneous endocytosis. Among suspension cells, lymphocytes only incidentally show cytoplasmic inclusions and monocytes degrade the particles by phagocytosis. The marked decrease in transfection efficiency generally observed between adherent and nonadherent cells is thus due to reduced cell **binding**. This suggests that cationic particles **bind** to membrane components responsible for Ca-2+-mediated cell anchoring to the extracellular matrix. Cation/anion-mediated endocytosis leads to endosomes that are entirely filled with the particles. Consequently, two escape mechanisms may operate: disruption of the lamellar envelope in close contact with tubular micelles, and endosome buffering by the lipopolyamine in response to proton entry, leading to osmotic swelling and endosome rupture. Even for moderately transfected MRC5 cells, 10-2-10-3 particles are found either free or in cytoplasmic vacuoles 24 h after transfection, highlighting a very inefficient nuclear translocation process. Such high numbers are also the clue to the small concentration window between transfection and cytotoxicity that is often observed with nonviral vectors. Nuclear particle inclusions are sometimes seen, yet it is unclear whether plasmid uncoating (before expression) takes place by anion exchange in the cytoplasm or in the nucleus. The still lower efficiency of free plasmid translocation to the nucleus suggests an active role for the **cationic lipid** during this step. Although the last stages of the transfection mechanism remain unclear, the present work shows that the major barrier which hampers in vitro gene delivery with cationic vectors is nuclear translocation (and cell entry for nonadherent cells), providing precise targets for the design of improved nonviral vectors.
ACCESSION NUMBER: 1996:575459 BIOSIS
DOCUMENT NUMBER: PREV199799290140
TITLE: An electron microscopy study into the mechanism of gene transfer with lipopolyamines.
AUTHOR(S): Labat-Moleur, F.; Steffan, A.-M.; Brisson, C.; Perron, H.; Feugeas, O.; Furstenberger, P.; Oberling, F.; Brambilla, E.; Behr, J.-P (1)
CORPORATE SOURCE: (1) Chimie Gene., Fac. Pharm., BP 24, 67401 Illkirch cedex France
SOURCE: Gene Therapy, (1996) Vol. 3, No. 11, pp. 1010-1017. ISSN: 0969-7128.
DOCUMENT TYPE: Article
LANGUAGE: English

L8 ANSWER 26 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
AB In a reporter gene assay, cationic liposomes containing the **cationic lipid** 3-beta-(N-(N',N'-dimethylaminoethane)carbamoyl)cholesterol (DC-Chol) and a neutral phospholipid dioleoylphosphatidylethanolamine (DOPE) showed high transfection activity. **DNA**/liposome complex which contained low amount of liposomes could **bind** to the cell surface but failed to transfect the cells. We have designed a two-step protocol to examine this

phenomenon in more detail. A431 human cells were incubated on ice (pulse) with **DNA** complexed to a low level of cationic liposomes. The cells were washed and incubated at 37 degree C (chase) with or without free cationic liposomes of various composition (helper liposomes). Only liposomes enriched with DOPE showed helper activity; liposomes containing dioleoylphosphatidylcholine (DOPC), a structural analog of DOPE, had no helper activity. The delivery was inhibited by the lysosomotropic agent chloroquine and was optimal if the helper liposome chase was initiated immediately after the pulse. An endocytosis model of **DNA** delivery by cationic liposomes is proposed in which the principal function of the chase liposomes is to destabilize the endosome membrane and allow the release of **DNA** into the cytosol. This model is consistent with the known activity of DOPE to assume non-bilayer structures, hence destabilizing the endosome membrane.

ACCESSION NUMBER: 1995:313935 BIOSIS
DOCUMENT NUMBER: PREV199598328235
TITLE: The role of dioleoyl phosphatidylethanolamine in cationic liposome mediated gene transfer.
AUTHOR(S): Farhood, Hassan; Serbina, Natalya; Huang, Leaf (1)
CORPORATE SOURCE: (1) Dep. Pharmacol., Univ. Pittsburgh Sch. Med., 13th Floor, Biomed. Sci. Tower, Pittsburgh, PA 15261 USA
SOURCE: Biochimica et Biophysica Acta, (1995) Vol. 1235, No. 2, pp. 289-295.
ISSN: 0006-3002.
DOCUMENT TYPE: Article
LANGUAGE: English

L8 ANSWER 27 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
AB Optimal in vitro gene delivery with cationic lipids requires an excess of cationic charges with respect to **DNA** phosphates. In these conditions, in vivo delivery will be hampered by interference from **cationic lipid-binding** macromolecules either circulating or in the extracellular matrix. To overcome this problem, we are developing a modular transfection system based on lipid-coated **DNA** particles reminiscent of enveloped viruses. The particle core consists of the lipopolyamine-condensed nucleic acid in an electrically neutral ratio to which other synthetic lipids with key viral properties are hydrophobically adsorbed. As a first result, we have found that a good transfection level can be achieved simply with the neutral core particle, provided a zwitterionic lipid (dioleoyl phosphatidylethanolamine) is added to completely coat the **DNA**. Addition of lipids bearing a fusogenic or a nuclear localization peptide head group to the particles does not significantly improve an already efficient system, in contrast to polylysine-based gene transfer methods that rely on lysosomotropic or fusogenic agents to be effective. This emphasizes the distinctive properties of the lipopolyamines, including cell membrane destabilization, endosome buffering capacity, and possibly nuclear tropism. Most importantly, addition of lipids with a triantennary galactosyl residue drives the neutral nucleolipidic particles to the asialoglycoprotein receptor of human hepatoma HepG2 cells: Transfection increases apprxeq 1000-fold with 25% galactolipid. This receptor-mediated process is saturable and slightly less efficient than receptor-independent transfection obtained in vitro with a large excess of **cationic lipid** alone. Yet, electrically silent particles may provide an attractive solution for gene transfer in vivo where their external saccharide coat should allow them to diffuse within the organism and reach their target cells.

ACCESSION NUMBER: 1995:206047 BIOSIS

DOCUMENT NUMBER: PREV199598220347
TITLE: [redacted]rgeted gene transfer into hepa[redacted]a cells with [redacted]ipopolyamine-condensed DNA particles presenting galactose ligands: A stage toward artificial viruses.
AUTHOR(S): Remy, Jean-Serge; Kichler, Antoine; Mordvinov, Vyatcheslav;
Schuber, Francis; Behr, Jean-Paul (1)
CORPORATE SOURCE: (1) Lab. Chimie Bioorganique, Unit. Recherche Associee Centre Natl. Recherche Sci., Fac. Pharmacie Strasbourg, route du Rhin, F-67401 Illkirch France
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1995) Vol. 92, No. 5, pp. 1744-1748.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English

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(FILE 'HOME' ENTERED AT 14:59:50 ON 19 MAR 2001)

FILE 'CAPLUS, BIOSIS' ENTERED AT 15:00:36 ON 19 MAR 2001
L1 1214 S CATIONIC (A) LIPID
L2 972 S CATIONIC (A) LIPOSOME
L3 972 S CATIONIC(A)LIPOSOME
L4 568 S DNA (S) L1
L5 366 S DNA (S) L2
L6 420 DUP REMOVE L4 (148 DUPLICATES REMOVED)
L7 1 S (DIMER? OR OLIGOMER?) (S) L6
L8 27 S (LINK? OR BIND?) (S) L6

=> s (thiol? or disulfide bridge? or cysteine) (s) l6

L9 0 (THIOL? OR DISULFIDE BRIDGE? OR CYSTEINE) (S) L6

=> d l6 py 1997

420 ANSWERS ARE AVAILABLE. SPECIFIED ANSWER NUMBER EXCEEDS ANSWER SET SIZE
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L6 ANSWER 1 OF 420 CAPLUS COPYRIGHT 2001 ACS
PY 2001

=> d l6 280-290 abs

L6 ANSWER 280 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB Today, nonviral gene transfer vectors attract more attention as a therapeutic strategy for human diseases, because viral vectors such as adenoviral and herpes viral vectors have been proven to have problems, esp. in immunogenicity and cytotoxicity. However, the main limitation of nonviral vectors has been low efficiency of gene expression. To overcome this defect, the authors have developed a new class of transfection vehicles, HVJ-cationic liposomes. The use of the **cationic lipid** DC-cholesterol facilitates efficient entrapment of neg. charged macromols. (plasmid **DNA**, oligodeoxynucleotides, and proteins) and efficient interaction with neg. charged plasma membranes of cultured cells in vitro. Moreover, the fusogenic envelope proteins of hemagglutinating virus of Japan (HVJ) enhance delivery of the enclosed

materials into cells. Using firefly luciferase as a marker, the authors optimized the [redacted]osome formula. As a result, t[redacted]authors have succeeded in obtaining 1[redacted]-800 times higher gene expression in vitro than with the conventional HVJ-anionic liposomes. However, in vivo gene transfer expts. have revealed that the use of cationic lipid instead of anionic lipid reduced the transgene expression dramatically in organs such as muscle and liver. The authors further discovered that the use of anionic liposomes with a viral-mimic king lipid compn. increased transfection efficiency by several times in vivo. The authors conclude that the alternative usage of transfer vectors, for example, HVJ-anionic liposomes for in vivo delivery to extended areas of organs and HVJ-cationic liposomes for in vitro delivery (and possibly for in vivo delivery to a restricted area of organs), is of significance.

L6 ANSWER 281 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB Physicochem. properties of the cationic liposomes, including structure of the cationic lipids, **cationic lipid**-to-**DNA** ratio, liposome particle size, and inclusion of the helper lipids, were studied for their effect on the level, site, and duration time of gene expression in vivo by i.v. administration. Using a cytomegalovirus (CMV)-driven gene expression system contg. either the luciferase or green fluorescence protein gene as a reporter and two cationic lipids [N-(2,3-dioleoyloxy)propyl-N,N,N-trimethylammonium chloride (DOTMA) and 1,2-dioleoyloxy-3-trimethylammonium propane (DOTAP)], we demonstrated in vivo by a single i.v. injection of DNA/liposome complexes into mice, that cationic liposomes are capable of transfecting genes in organs such as the lung, heart, liver, spleen, and kidney. Transfection efficiency is detd. mainly by the structure of the **cationic lipid** and the ratio of **cationic lipid** to **DNA**. Although the presence of cholesterol in DOTAP liposomes did not affect transfection activity, inclusion of dioleoylphosphatidylethanolamine (DOPE) into either DOTAP or DOTMA liposomes significantly decreases liposome transfection activity in vivo. Results from time course show that gene expression in different organs is transient, with a peak level between 4 and 24 h, dropping to less than 1% of the peak level by day 4. Expts. with repeated injections showed that the peak level of gene expression could be regained by subsequent injection.

L6 ANSWER 282 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 104
AB Cationic lipids show promise as vectors for transfer of CFTR cDNA to airway epithelia of patients with cystic fibrosis (CF). However, previous studies have not compared the effect of DNA-lipid to DNA alone. Recently, the authors developed a formulation of plasmid encoding CFTR (pCF1-CFTR) and **cationic lipid** (GL-67:DOPE) that generated greater gene transfer in mouse lung than previously described **DNA**-lipid vectors. Therefore, the authors tested the hypothesis that DNA-lipid complexes were more effective than DNA alone at transferring CFTR cDNA to airway epithelia in vivo. The authors administered complexes of DNA-lipid to one nostril and DNA alone to the other nostril in a randomized, double-blind study. Electrophysiol. measurements showed that DNA-lipid complexes partially cor. the Cl- transport defect. Importantly, the pCF1-CFTR plasmid alone was at least as effective as complexes of DNA with lipid. Measurements of vector-specific CFTR transcripts also showed gene

transfer with both DNA-lipid and DNA alone. These results indicate that nonviral vectors can transfer CFTR cDNA to airway epithelia and at least partially restore the Cl- transport defect characteristic of CF. However, improvements in the overall efficacy of gene transfer are required to develop a treatment for CF.

L6 ANSWER 283 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 105
AB Complexes of **DNA** and **cationic lipid** offer potential advantages for gene transfer to airway epithelia. However, we found that application of DNA-lipid (DMRIE-DOPE) complexes o primary cultures of human ciliated airway epithelia or explants of rabbit trachea generated only low levels of gene transfer. In contrast, when we applied the DNA-lipid to immature human epithelia shortly after seeding, transgene expression was substantially higher. We identified two barriers that limit gene transfer. First, uptake of the DNA-lipid complexes into airway cells across the apical membrane decreased rapidly with time after seeding and paralleled the decrease in transgene expression. Second, cell division decreased with time after seeding, and we found that cells in mitosis (labeled with BrdU) were much more likely to express transgene than BrdU-neg. cells. These data suggest that the entry step across the apical membrane and the low rate of cell division are important barriers for cationic lipid-mediated gene transfer to airway epithelia. Attempts to modify these two processes may yield improvements in the efficiency of gene transfer to the airways in cystic fibrosis.

L6 ANSWER 284 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS
AB Novel, double-chained pyridinium compounds have been developed that display highly efficient **DNA** transfection properties. The transfection efficiency of several of these compounds is enhanced by an order of magnitude, when compared with the transfection efficiency accomplished with the widely used **cationic lipid** system, lipofectin. Most importantly, the pyridinium compounds were found to be essentially nontoxic toward cells. Using various reporter genes, such as beta-galactosidase and pNEO (a gene construct that renders cells resistent to antibiotic derivatives of neomycin like G418), we demonstrate that the enhanced efficiency relates to the fact that a relative higher number of cells in the population is transfected (apprxeq 50% in the case of COS cells) by the pyridinium derivatives, whereas the delivery of **DNA** per cell is also enhanced. Furthermore, application of the pyridinium derivatives shows little cellular preference in their ability to transfect cells. By systematically modifying the structure of the pyridinium amphiphile, i.e., by changing either the headgroup structure or the alkyl chains, some insight was obtained that may lead to unraveling the mechanism of amphiphile-mediated transfection, and thus to protocols that further optimize the carrier properties of the amphiphile. Our results reveal that unsaturated alkyl chains enhance the transfection properties of the pyridinium-based amphiphiles. Preliminary experiments suggest that the structure-dependent improvement of transfection efficiency, when comparing pyridinium derivatives with lipofectin, likely relates to the mechanism of delivery rather than the packaging of the amphiphile/**DNA** complex.

L6 ANSWER 285 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

L6 ANSWER 286 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 106
AB We compared the efficacy of gene transfer in vitro and in vivo using various formulations of **DNA**-lipid complexes based on the novel **cationic lipid** EDMPC (1,2-dimyristoyl-sn-glycero-3-

ethylphosphocholine, chloride salt). In vitro studies analyzed delivery of marker gene to four established cell lines, including two of pulmonary origin. The in vivo anal. used intralobar delivery of marker genes and CFTR to mice and rats. We obsd. a lack of pos. correlation between those DNA-EDMPC formulations that delivered DNA most efficiently in vitro and those that worked best in vivo. Intralobar DNA delivery to rodents mediated by EDMPC was efficient. The high level of gene delivery by DNA-EDMPC formulations demonstrates that efficient lipid-mediated gene transfer to the lung is possible.

L6 ANSWER 287 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 107

AB A novel LPD formulation has been developed for in vivo gene transfer. It involves the interaction of plasmid DNA with protamine sulfate, a cationic polypeptide, followed by the addn. of 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP) cationic liposomes. Compared with DOTAP/DNA complexes, LPD offers better protection of plasmid DNA against enzymic digestion and gives consistently higher gene expression in mice via tail vein injection. When a luciferase reporter gene was employed, gene expression was found in all tissues examd. including lung, heart, spleen, liver and kidney with the highest expression in the lung. The in vivo efficiency of LPD was dependent upon charge ratio and was also affected by the lipid used. Increasing the amt. of DNA delivered induced an increase in gene expression. The optimal dose was approx. 50 .mu.g per mouse at which concn. approx. 20 ng luciferase protein pere milligram extd. tissue protein could be detected in the lung. Increasing, the DNA to 100 .mu.g per mouse resulted in toxicity and death of the animal. Gene expression in the lung was detected as early as 1 h after injection, peaked at 6 h and declined thereafter. High expression was also found in the spleen 6 h after injection but dropped very rapidly thereafter. The in vivo gene expression by LPD was dependent upon the route of administration since intraportal injection of LPD led to about a 100-fold decrease in gene expression in the lung as compared with i.v. injection. Using lacZ as a reporter gene, it was shown that endothelial cells were the primary locus of transgene expression in both the lung and spleen. No sign of inflammation in these organs was noticed. Since protamine sulfate has been proven to be nontoxic and only weakly immunogenic in humans, this novel vector may be useful for clin. gene therapy.

L6 ANSWER 288 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

AB LMH-2A is an estrogen-responsive avian hepatoma cell line whose susceptibility to **cationic-lipid**-mediated transfection is poorly described. 3-beta(N-N',N'-dimethylaminoethane)-carbamoyl) cholesterol (DCC) requires a one-step synthesis, and can be used to formulate transfection-grade liposomes when combined with dioleoylphosphatidyl-ethanolamine (DOPE) 1/1 (wt/wt). Luciferase activities in LMH-2A cells were 8.5-fold and 87.5-fold greater than those in HepG2 and FTO2B cells, respectively, following DCC-liposome-mediated transfection with a reporter consisting of the human cytomegalovirus immediate-early promoter (CMV), joined to Photinus pyralis luciferase (L) cDNA, designated pCMVL. Using pCMVL, N-(2-bromoethyl)-N,N-dimethyl-2,3-bis(9-octadecenyloxy)-propanaminimun bromide) (BMOP)/DOPE 1/1 (wt/wt), at a 7.5:1 ratio with **DNA**, produced luciferase activities that were 2.9-fold higher than those of DCC-liposomes, at an optimal 10:1 lipid: **DNA** ratio. At optimal lipid:**DNA** ratios, commercially available liposomes, Transfectam, Lipofectamine, and Lipofectin, produced luciferase activities that were 1.39, 1.03, and 0.47-fold those of DCC-liposomes. The effect of 0, 10, 100, or 500 nM/L 17-beta-estradiol on the expression of pCMVL and a second luciferase reporter containing the

-593/+48 promoter region of the estrogen-responsive avian apo VLDL-II gene, designated pApoL, was tested in cells cultured in the presence or absence of 10% chicken serum. The CMV promoter supported a high level of expression in LMH-2A cells that was unaffected by serum alone, but was weakly responsive to estrogen. Estrogen responses of both reporters reached a plateau at 10 nM/L. Estrogen increased the expression of pApoL 24-fold and 79-fold in the absence and presence of serum, respectively. The -593/+48 region of the apo VLDL-II promoter may not contain previously reported negative insulin response elements, but chicken serum contains factors that enhance estrogen responsiveness of this region.

L6 ANSWER 289 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 108
AB Optimizing gene expression and delivery are necessary steps in the prodn. of vectors for DNA-based immunization as well as for other gene therapy applications. A mouse muscle/reporter gene assay system was used to systematically improve a plasmid DNA vector. The optimized vector VR1255 contained: (1) CMV promoter and enhancer; (2) CMV IE Intron A; (3) kanamycin resistance gene; (4) deleted SV40 origin of replication; (5) optimized lux coding region; and (6) a minimal synthetic terminator from the rabbit beta globin gene, mRBG. The vector VR1255 expressed 137 times greater than an earlier prototype RSV-based vector. For plasmid vector delivery into nonmuscle tissues, a recently synthesized **cationic lipid**, GAP-DLRIE, was found to greatly enhance the uptake and expression of plasmid **DNA** by 100-fold when instilled into the mouse lung. The time-course of CAT expression with GAP-DLRIE indicated that peak expression occurs 2-5 days after intranasal administration and expression diminished to about one-third the peak value by day 21. This cationic lipid may be useful for immunization by pulmonary and perhaps other nonmuscle routes.

L6 ANSWER 290 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB Advances in gene therapy vectors and techniques hold promise for treatment of many inherited and acquired diseases. For lung indications, esp. those involving the epithelium, delivery of the gene therapy vehicle ideally will involve the use of an aerosol. Aerosol delivery of transgenes using cationic lipids is currently limited by the ability to generate highly concd. formulations of lipid:DNA complexes that are stable and retain their activity following aerosolization. We have examd. many of the variables inherent in aerosolizing cationic lipid gene delivery vehicles and have devised a new formulation that incorporates small amts. of a polyethylene glycol-contg. lipid. This formulation has allowed the prepn. of concd. dispersions of **cationic lipid**:plasmid **DNA** (pDNA) complexes (>20 mM pDNA) at approx. 10-fold higher concns. than previously reported. Most of the pDNA in these formulations was bound to the lipid component and thereby protected from nebulizer-induced shearing; the pDNA also maintained full biol. activity both in vitro and in vivo. This new formulation thus represents a significant improvement over current methods to prep. concd., active cationic lipid gene delivery vectors, and provides a new tool with which to test gene transfer to the lung.

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L6 ANSWER 291 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 109

AB A stable **cationic lipid/DNA** complex has been developed for in vivo gene transfer. The formulation capitalizes on a previously described procedure to obtain stable lipid/DNA complexes for in vitro gene transfer (1). Conditions for DNA/lipid complex formation were modified to yield a DNA concn. of 1 mg/mL. Heat stable alk. phosphatase (AP) under a CMV promoter was used as a reporter gene. The resulting complex was completely insensitive to serum inactivation. Tail vein injection of a 80 .mu.g DNA into Balb C mice yielded significant levels of reporter enzyme activity in the lung, heart, spleen, muscle, and liver. Less AP activity was obsd. in the kidney. No AP activity was obsd. in blood, bone marrow or brain. A titrn. of the lipid (DOSPA) to DNA-nucleotide ratio showed the optimal molar ratio for in vivo gene transfer to be 1/1. Using this ratio in a dose response study showed approx. 80.mu.g of DNA/mouse yielded the highest level of gene expression. Using this dose at a 1/1 lipid to DNA nucleotide ratio, the time course for alk. phosphatase activity was detd. Maximal AP activity was obsd. 24 h after injection for all tissues. By day 5, the activity dropped approx. 10 fold for all tissues. By day 7, residual activity was detected in the lung, heart, and muscle. Histol. of the lung showed both interstitial and endothelial cells to be transfected. In all other tissues, however, endothelial cells were the only transfected cell type. These results demonstrate that reformulation of an existing **cationic lipid** can result in the formation of a stable lipid/**DNA** complex, which is able to reproducibly transfect lung, heart, spleen, and liver upon i.v. administration.

L6 ANSWER 292 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

AB Complexes of **DNA** and cationic lipids are promising vectors for gene transfer. Most **cationic lipid** formulations contain both a cationic component and a neutral co-lipid. We found that the co-lipid could influence **DNA** uptake in COS-1 cells, but processes subsequent to uptake were even more important in determining gene expression. We compared dioleoylphosphatidylethanolamine (DOPE) and structural analogs of DOPE combined with cationic lipids and found that **DNA** uptake and transgene expression did not always correlate. Transgene expression was dependent on **DNA** uptake into the cell, on entry of **DNA** into the cytoplasm, and on release of **DNA** from the lipid complex. We found that some co-lipids had a greater effect on **DNA** uptake, whereas others had a greater effect on steps subsequent to entry. Based on these results, we tested the

hypothesis that co-lipids conferring different properties could be combined to enhance gene transfer. The results showed that a combination of co-lipids had a synergistic effect on expression. We also found that structural analogs of DOPE were more effective than DOPE in enhancing gene transfer to mature human airway epithelia studied in vitro and to mouse lung studied in vivo. These data provide insight into the mechanism by which co-lipids influence **cationic lipid**-mediated gene transfer and show that optimization of the effects of co-lipids can enhance gene transfer both in vitro and in vivo.

L6 ANSWER 293 OF 420 CAPLUS COPYRIGHT 2001 ACS

AB Studies have indicated that although abundant levels of transgene expression could be achieved in the lungs of mice instilled with cationic lipid:pDNA complexes, the efficiency of gene transfer is low. As a consequence, a relatively large amt. of the complex will need to be administered to the human lungs to achieve therapeutic efficacy for indications such as cystic fibrosis. Because all cationic lipids exhibit some level of cytotoxicity in vitro, the authors assessed the safety profile of one such cationic lipid, GL-67, following administration into the lungs of BALB/c mice. Dose-dependent pulmonary inflammation was obsd. that was characterized by infiltrates of neutrophils, and, to a lesser extent, macrophages and lymphocytes. The lesions in the lung were multifocal in nature and were manifested primarily at the junction of the terminal bronchioles and alveolar ducts. The degree of inflammation abated with time and there were no apparent permanent fibrotic lesions, even in animals that were treated at the highest doses. Anal. of the individual components of the complex revealed that the pulmonary inflammation was primarily cationic lipid-mediated with a minor contribution from the neutral co-lipid DOPE. Assocd. with the lesions in the lungs were elevated levels of the pro-inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor-.alpha. (TNF-.alpha.), and interferon-.gamma. (IFN-.gamma.) that peaked at days 1-2 post-instillation but resolved to normal limits by day 14. Total cell counts, primarily of neutrophils, were also significantly elevated in the bronchoalveolar lavage fluids of GL-67:pDNA-treated mice between days 1 and 3 but returned to normal limits by day 14. No specific immune responses were detected against the **cationic lipid** or plasmid **DNA** in mice that had been either instilled or immunized with the individual components or complex, nor was there any evidence of complement activation. These studies indicate that a significant improvement in the potency of cationic lipid:pDNA formulations is desirable to minimize the toxicity assocd. with cationic lipids.

L6 ANSWER 294 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 110

AB The factors controlling the transfection efficiency of cationic lipid carrier systems following i.v. administration are poorly understood. Using N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) combined with Tween 80 as a carrier system and cDNA of luciferase or .beta.-galactosidase gene as a reporter, we investigated the importance of DOTMA to DNA ratio and the ratio of DOTMA to Tween 80 in the lipid formulation in detg. the site and level of transgene expression following i.v. administration. The data show that all of the internal organs, including lung, liver, spleen, heart and kidneys, expressed the transgene upon systemic administration into animals with 25 .mu.g of plasmid DNA when complexed with DOTMA-Tween 80 lipid formulation. The transfection efficiency was dependent on both DOTMA to DNA, and DOTMA to Tween 80 ratios. Among the organs examd., the lung appeared to be more transfectable than other organs. A better transfection activity was obtained with higher DOTMA to DNA and DOTMA to Tween 80 ratios. Time-response curve shows that gene expression was transient with a

maximal level between 10 and 24 h after injection. Results from tissue distribution studies with 125I-labeled plasmid DNA and Southern anal. suggest that the transient expression is the result of the loss of transgene from the transfected cells. These results suggest that **cationic lipid**-based delivery systems ban be efficient for gene delivery if the compn. of the **DNA**-lipid complexes is properly controlled.

L6 ANSWER 295 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB **DNA** mols. adsorbed onto supported **cationic lipid** membranes are highly condensed. The 2-D condensation requires the presence of free DNA mols. in the soln. for a period about 12 h, in which the ratio of adsorbed DNA to the free DNA is about 5% or less. Once condensed, the adsorbed DNA mols. remain on the membrane after removing free DNA mols. in the soln. The adsorption-condensation of both the linear and the circular forms of DNA onto the cationic membrane is independent of the length of DNA. For the condensed DNA, the interhelical distance increases with increase in the concn. of monovalent Na+ ions. The fluidity of the membrane is essential to induce the close packing of the adsorbed DNA. Possible mechanisms of our findings are discussed.

L6 ANSWER 296 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB **Cationic lipid**-mediated transfection of the alveolar epithelium in vivo will require exposure of plasmid **DNA** and cationic lipids to endogenous surfactant lipids and proteins in the alveolar space. Effects of pulmonary surfactant and of surfactant constituents on transfection in vitro of two respiratory epithelial cells lines (MLE-15 and H441) with a plasmid encoding the luciferase reporter gene were studied using two cationic lipid formulations: 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide/cholesterol (DMRIE/C) and 1,2-dioleoyl-3-trimethylammonium propane/dioleoyl phosphatidylethanolamine (DOTAP/DOPE). Gene expression, as assessed luciferase activity, decreased as increasing concns. of natural surfactant were added to **cationic lipid-DNA** complexes. Incorporation of phospholipids DOPC/DOPG or surfactant proteins SP-B or SP-C in the cationic lipid formulation inhibited transfection. A fluorescent lipid mixing assay was used to det. the effects of surfactant proteins SP-B and SP-C on mixing between **cationic lipid-DNA** complexes and surfactant lipid vesicles. Mixing between DOPC/DOPG vesicles and **cationic lipid-DNA** complexes in the absence of added proteins amounted to 10-20%. Addn. of SP-B or SP-C increased the mixing of DOPC/DOPG vesicles with DOTAP/DOPE-DNA complexes, but not DMRIE/C-DNA complexes. These results demonstrate that pulmonary surfactant lipids and proteins inhibit transfection with **cationic lipid-DNA** complexes in vitro, and may therefore represent a barrier to gene transfer in the lung.

L6 ANSWER 297 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

L6 ANSWER 298 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB Allovectin-7 is a gene therapy agent that consists of plasmid **DNA** (pDNA) encoding the human HLA-B7 class I and .beta.2-microglobulin genes (VCL-1005), complexed with the **cationic lipid** DMRIE Br and DOPE. A tritiated version of the cytofectin component, DMRIE Br, was synthesized by regiospecific isotope incorporation to very high specific activity. The 3H-labeled DMRIE/DOPE mixt. was complexed with VCL-1005 to produce a radiolabeled version of Allovectin-7. The VCL-1005/3H-DMRIE/DOPE complex was administered i.v. to mice, and the tissue distribution of radioactivity was analyzed 24 h later. Excretion of

radioisotope was monitored for 96 h post dosing. At 24 h post administration, tissue distribution for the radioisotope of liver >> spleen > lung > heart > brain .apprxeq. muscle .apprxeq. blood was obsd. During the 96-h period post dose, very little administered radioactivity (<17%) was excreted and the majority of the isotope (83%) remained in the animal. This is the first report on the biodistribution of the cytofectin component of a pDNA-cationic lipid complex which the distribution of the plasmid component has also been reported.

L6 ANSWER 299 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

AB Purpose: To determine the safety, toxicity, and efficacy of direct intratumoral injection of an allogeneic major histocompatibility complex (MHC) class I gene, HLA-B7, in a **cationic lipid** vector (Allovectin-7; Vical Inc, San Diego, CA) in patients with metastatic melanoma. Patients and Methods: Seventeen HLA-B7-negative patients were treated with intralesional injection of Allovectin-7. Twelve patients received a single intralesional injection containing 10 mu-g (four patients), 50 mu-g (five patients), or 250 mu-g (three patients) of plasmid **DNA**. Five patients received two or three injections of 10 mu-g **DNA** to a single tumor site at 2-week intervals. Tumor biopsies pretherapy and 2 and 4 weeks after gene injection were obtained to determine expression of the plasmid by polymerase chain reaction (PCR), reverse transcriptase (RT)-PCR, flow cytometry, and immunohistochemistry. Results: Toxicities were related to technical aspects of the injections or biopsies. These included pain, hemorrhage, pneumothorax, and hypotension. Two patients were hospitalized overnight for observation. Seven patients (50%) had tumor responses insofar as the injected nodule decreased gtoreq 25% by radiologic or physical examination. One patient with a single site of disease achieved a complete remission. Ninety-three percent of the patients' post-gene therapy biopsies contained HLA-B7 plasmid **DNA**, mRNA, or protein. Conclusion: Intratumoral injection of the allogeneic histocompatibility gene, HLA-B7, in a lipid vector can be performed safely at plasmid **DNA** doses ltoreq 250 mu-g. The safety profile and biologic activity of this therapy warrants further studies to define the mechanism of action, predictors of response, and antitumor efficacy of this approach.

L6 ANSWER 300 OF 420 CAPLUS COPYRIGHT 2001 ACS

AB We have examd. several variables inherent in aerosolizing **cationic lipid:DNA** complexes using a jet nebulizer and thereby have optimized the delivery of functional complexes. Maximal aerosol transfer efficiency of cationic lipid:pDNA complexes was quantitated and shown to require the presence of at least 25 mM NaCl as an excipient. This is possibly related to effects on the measured zeta potentials of the complex, which indicate that the complexes are more highly charged in solns. of physiol. ionic strength than in solns. of low ionic strength. Inclusion of saline also resulted in retention of the starting lipid to plasmid DNA (pDNA) ratio following nebulization. These data were used to design in vitro aerosolization expts. with tissue culture cells that resulted in the identification of a cationic lipid:pDNA ratio of 0.75:1 (mol:mol) as being optimal for aerosolization. This formulation largely protected pDNA from shear degrdn. during nebulization and produced a respirable aerosol droplet size (1-3 .mu.m). It was tested further in a mouse model and shown to result in the dose-dependent transfection of mouse lungs, generating the equiv. of several picograms of reporter gene activity per mouse lung. The results of these expts. have provided a set of optimal conditions for nebulizing cationic lipid:pDNA complexes that can be used as a starting point for the further evaluation of aerosol delivery of these nonviral gene delivery vectors in vivo.

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L6 ANSWER 300 OF 420 CAPLUS COPYRIGHT 2001 ACS
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L6 ANSWER 301 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB Lipofection has become a simple, effective and safe method to introduce DNA, RNA and proteins into cells. One of the cationic liposome formulations developed by us, DC-chol/DOPE liposomes, is relatively non-toxic and efficient in transfection. Recently, this liposome formulation has been used in two sep. clin. trials for the immunotherapy of malignancy and for treatment of cystic fibrosis genetic defects. The structure of DNA/liposome complexes and their mode of action in transfection will be reviewed. Based on this information, two types of novel condensed structure contg. DNA polycation and lipids have been developed. The LPD (lipid-entrapped, polycation-condensed DNA) particles are small (<100 nm in diam.), monodisperse, and colloidally stable. The transfection activity of LPD is similar to that of adenovirus and is about 10-100 fold higher than that of the first generation cationic liposomes. The LPD I particles are cationic and used primarily in local and regional delivery routes. The LPD II particles are anionic and can be target specific by attaching specific ligand mols. on the surface. The parenteral use of these novel particles for systemic gene transfer is under development. Recently, reconstituted chylomicron remnants have been used to solubilize **DNA/cationic lipid** complexes. This new non-viral vector induced high level transgene expression in the liver. These formulations will be discussed in terms of their efficiency, toxicity and potential uses in gene therapy.

L6 ANSWER 302 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB The interaction between large duplex T4 **DNA** (166 kbp) and a synthetic dialkyl **cationic lipid,** distearyldimethylammonium bromide (D18DAB), was studied using fluorescence microscopy for single-chain observation. The dependence of the higher-order structure of single T4 DNAs on the surfactant concn. was evaluated, starting at extremely low values (1.0 .times. 10-8 M). Individual T4 DNA chains undergo a marked discrete transition between elongated coil and compact globule states, and there is a very wide region

of coexistence (about two orders of magnitude of the surfactant concn.) for the coiled and globular T4 DNAs. We propose a simple theor. model for assessing the relationship between the binding equil. and the coil-globule transition. In addn., liposomes composed of neutral phospholipids induce unfolding of DNA compacted by a cationic surfactant.

L6 ANSWER 303 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB A review with 30 refs. When cationic liposomes are mixed with plasmid DNA an aggregation reaction occurs. The heterogeneous membrane structures which arise following this reaction are dependent on liposomal lipid compn., liposome/DNA ratio as well as the presence of added salts or proteins. The resulting structures are also unstable, resulting in time dependent changes in the phys. attributes of the aggregates. Pharmaceutical development of the liposome/DNA aggregates will be challenging because of these factors. For these reasons we have pursued development of alternative lipid-based systems as a vehicle for gene transfer. The pivotal step that led to development of these novel systems was the identification and isolation of a hydrophobic **cationic lipid/DNA** complex. This complex can be used as an intermediate in the prepn. of well-defined particles. The hydrophobic lipid/DNA complex and the liposome/DNA aggregates are both formed as a consequence of multivalent electrostatic interactions. In contrast to the liposome/DNA aggregates, however, we believe that the particles formed when using lipid/DNA complex intermediates are a consequence of hydrophobic interactions.

L6 ANSWER 304 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 111
AB The authors have characterized a new synthetic gene delivery system, termed DLS, which may be suitable for systemic gene therapy. DLS constitutes a lipopolyamine and a neutral lipid and assocd. plasmid DNA in the formation of lamellar vesicles (DLS-DNA). The ratio of lipids and lipid to **DNA** as well as the method of prepn. were optimized to yield a high in vitro transfection efficiency compared with that previously reported for **cationic lipid** systems. DLS-DNA showed a rapid cellular uptake and distribution in the cytoplasmic and nuclear (esp. in the nucleoli) compartments as detd. by laser-assisted confocal microscopy. There was little or no plasmid DNA degrdn. over a period of 20 min, relatively slow plasma clearance, and effective and rapid cellular uptake of DLS-DNA following i.v. administration in mice. Supercoiled plasmid DNA could be detected in blood cells .ltoreq.1 h after injection. Systemic administration of DLS-DNA yielded transgene expression in mouse tissues, such as in lung or liver. The ratio of DLS:DNA and the procedure used to form DLS-DNA affected both the level and cellular specificity of expression of a luciferase reporter gene showing that in vitro transfection efficiency of DLS-DNA formulations cannot be easily extrapolated to an in vivo setting. Optimization of the formulation of a DNA delivery system was crit. to obtain a defined structure resulting in a prepn. with high reproducibility and stability, greater homogeneity of particle size and high efficacy following systemic gene transfer. In addn., the DLS system may be formulated for specific target tissues and may have a wide range of applications for gene therapy.

L6 ANSWER 305 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS
AB Addition of cationic lipids to plasmid **DNA** considerably

increases the efficiency of transfection. The mechanism has not yet been elucidated. A possibility is that these compounds destabilize biological membranes (plasma, endosomal, lysosomal), facilitating the transfer of nucleic molecules through these membranes. We have investigated the problem by determining if a **cationic lipid** N-(1-(2,3-dioleoxy)propyl)-N,N,N,-trimethylammonium methyl-sulfate (DOTAP, Boehringer, Mannheim, Germany) affects the integrity of rat liver lysosomal membrane. We have measured the latency of beta-galactosidase, a lysosomal enzyme, and found that incubation of lysosomes with low concentrations of DOTA.P causes a striking increase in free activity of the hydrolase and even a release of the enzyme into the medium. This indicates that lysosomal membrane is deeply destabilized by the lipid. The phenomenon depends on pH, it is less pronounced at pH 5 than at pH 7.4. Anionic compounds, particularly anionic amphipathic lipids, can to some extent prevent this phenomenon. It can be observed with various cationic lipids. A possible explanation is that cationic liposomes interact with anionic lipids of lysosomal membrane, allowing a fusion between the lipid bilayers which results in a destabilization of the organelle membrane.

L6 ANSWER 306 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

AB Purpose. We have demonstrated that a heteromolecular complex consisting of cationic lipids and **DNA** can be prepared and isolated (I). Cationic lipids bind **DNA** through electrostatic interactions. However, when sufficient lipids are bound to **DNA** the physical and chemical properties of the complex are governed by hydrophobic effects. Here we describe an approach where this hydrophobic complex is used as an intermediate in the preparation of lipid-**DNA** particles (LDPs). Methods. The approach relies on the generation of mixed micelles containing the detergent, n-octyl beta-D-glucopyranoside (OGP), the **cationic lipid**, N-N-dioleoyl-N, N-dimethylammonium chloride (DODAC), and selected zwitterionic lipids, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) or egg sphingomyelin (SM). Results. When these micelles were prepared at low detergent concentrations (20 mM OGP) and combined with pCMV-beta **DNA**, LDPs spontaneously formed. The mean diameter of these particles as measured by quasielastic light scattering was 55-70 nm, a result that was confined by negative stain electron microscopy. Further characterization of these LDPs showed that **DNA** within the particles was inaccessible to the small fluorochrome TO-PRO-1 and protected against DNase I degradation. LDPs could also be prepared in high concentrations of OGP (100 mM), however particles formed only after removal of OGP by dialysis. Particles formed in this manner were large (gt 2000nm) and mediated efficient transfection of Chinese hamster ovary cells. Transfection activity was greater when the lipid composition used consisted of SM/DODAC. Small particles (lt 100nm) prepared of SM/DODAC were, however, inefficient transfecting agents. Conclusions. We believe that LDP formation is a consequence of the molecular forces that promote optimal hydrocarbon-hydrocarbon interactions and elimination of the hydrocarbon-water interface.

L6 ANSWER 307 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 112

AB Noticeable modifications of in-serum transfection efficiency of dioctadecylamidoglycyl-spermine (DOGS)-DNA complexes are obsd., depending on DNA condensation conditions. The structures of the complexes are studied, keeping in mind the variability of lipid polymorphism, by cryo-TEM and x-ray diffraction. By increasing both pH and ionic strength, well-organized lamellar structures with a period of 65 .ANG. replace supramicellar aggregates. A relation between the structures and their in-vitro transfection activity is established. Efficiency in the presence

of serum is maintained when a lamellar arrangement is involved.

L6 ANSWER 308 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 113
AB A 32P-labeled pCMV-CAT plasmid DNA was used to est. the DNA uptake efficiency and unlabeled pCMV-CAT plasmid DNA to quantify the CAT activity after transfection of COS cells using each of the 3 following cationic compds.: vectamidine (3-tetradecylamino-N-tert-butyl-N'-tetradecylpropionamidine, and previously described as diC14-amidine), lipofectin (a 1:1 mixt. of N-(1-2,3-dioleyloxypropyl)-N,N,N-triethylammonium (DOTMA) and dioleylphosphatidylethanolamine (DOPE)), and DMRIE-C (a 1:1 mixt. of N-[1-(2,3-dimyristyloxy)propyl]-N,N-dimethyl-N-(2-hydroxyethyl) ammonium bromide (DMRIE) and cholesterol). Surprisingly, a high CAT activity was obsd. with vectamidine although the DNA uptake efficiency was lower as compared to lipofectin and DMRIE-C. Transmission electron microscopy (TEM) revealed endocytosis as the major pathway of **DNA-cationic lipid** complex entry into COS cells for the 3 cationic lipids. However, the endosomal membrane in contact with complexes contg. vectamidine or DMRIE-C often exhibited a disrupted morphol. This disruption of endosomes was much less frequently obsd. with the DNA-lipofectin complexes. This comparison of the 3 compds. demonstrates that efficient transfection mediated by cationic lipids is not only correlated to their percentage of uptake but also to their ability to destabilize and escape from endosomes.

L6 ANSWER 309 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 114
AB Cationic liposomes have been studied as a potential carrier for delivering genes to cells for the purpose of gene therapy. This report summarizes our efforts to characterize the in vivo expression of transgene delivered by cationic liposomes via i.v. administration. Using a CMV driven gene expression system contg. cDNA of luciferase or green fluorescence protein gene as a reporter and two commonly used cationic lipids, 2,3-dioleoyloxypropyl-1-trimethylammonium chloride (DOTMA) and 2, 3-dioleoyloxyl-1-trimethylammoniumpropanoyl chloride (DOTAP), we demonstrate that a significant level of gene expression can be obtained in different organs including the lung, heart, spleen, liver and kidneys following i.v. administration in the mouse. Our finding show that the transfection efficiency of cationic liposomes is detd. by the structure of the cationic lipids, the lipid compn. of liposomes and **cationic lipid** to **DNA** ratio. Furthermore, gene expression was short in duration, peaked between 4-24 h post injection, and dropped to less than 1% of the peak level within a 4 day period. Expts. with repeated injections revealed that cells initially transfected by the first transfection were not fully responsive to the subsequent second transfection for approx. 14 days.

L6 ANSWER 310 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 115
AB Efficient transfection conditions for a no. of human, rat and rabbit primary cells and established lines of vascular origin have been detd. using a complex of a com. available cationic lipid transfection agent (TfxTM-50) and luciferase reporter plasmid constructs. The optimized conditions have also been successfully applied to rabbit carotid arteries in vivo ad a series of human arteries in vitro. The most crit. factors influencing the efficiency of gene transfection with this protocol are: DNA concn.; ratio fo lipid reagent to DNA; transfection time and the presence or absence of serum. Immunohistochem. anal. shows that a high percentage of cells (approx. 30-80% dependent on lineage) were transfected under optimal conditions with minimal toxicity effects. Similar analyses

performed on undamaged rabbit carotid vessels transfected in vivo and human arteries transfected in vitro show high-efficiency transfer and strong expression of the luciferase vector as demonstrated by reporter gene expression. The optimization of gene transfer into vascular cells with this cationic lipid complex will be valuable for mol. studies of genes implicated in cardiovascular diseases and as a possible method of gene delivery with therapeutic intent.

L6 ANSWER 311 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

L6 ANSWER 312 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 116
AB Cationic lipids are widely used for gene transfer into cultured eukaryotic cells. However, lipids with potent transfection activity are often assocd. with high levels of cytotoxicity, and also require serum-free conditions for optimal performance. These characteristic in many cases result in unsatisfactory transfection efficiency. In this report, the authors describe a new cationic amphiphile, N-t-butyl-N'-tetradecyl-3-tetradecylaminopropionamide (Amidine). Amidine requires only 1-2 h incubation intervals to produce maximal transfection efficiency, and can transfect cells in the presence of serum. Such characteristics significantly minimize cytotoxicity, and also provide time flexibility for researchers. The authors routinely obtain over 80% transfection efficiency as evidence by use of an enhanced green fluorescence protein (EGFP) as the reporter. These studies demonstrate the utility of Amidine for rapid and efficient transfection of mammalian cells.

L6 ANSWER 313 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB A review with 127 refs. Fluorescence microscopy technique is applied for the study of conformational changes of double-stranded DNA mols. induced by surfactants in aq. soln. The interaction of large T4DNA mols. with cationic surfactant, cetyltrimethylammonium bromide (CTAB) and synthetic dialkyl **cationic lipid**, distearyldimethylammonium bromide (D18DAB), is studied using fluorescence microscopy for the single-chain observation of **DNA** chains. It is found that large DNA mols. exhibit discrete conformational change between coil and globule states with the addn. of CTAB. To study the dynamical aspect of coil-globule transition, translational diffusion const. and hydrodynamic gyration radius of DNA mols. are measured from the time-series of video frames of the fluorescence image. The interaction between CTAB and T4DNA is also studied by potentiometric titrn. with the use of ion-selective membrane electrode. The data obtained are discussed in comparison with the results on the higher order structure of T4DNA with the addn. of CTAB. It is shown that the apparent cooperativity represented as a sigmoidal function is attributed to the bimodality in the distribution between elongated coil and compact globule states of T4DNA chains. A simple theor. model for assessing the relationship between the binding equil. and the coil-globule transition is proposed instead of the one-dimensional model for the sigmoidal transition. It is also shown that the globule-coil transition of single T4DNA is reversible as is detected by single mol. observation with fluorescence microscopy. The synthetic polyelectrolytes and liposomes composed of neutral phospholipids are found to induce unfolding of DNA compacted by a cationic surfactant. It is also indicated that at some crit. low-mol.-wt. salt concn. surfactant ions do not induce the DNA collapse. This effect, attributed to the screening of neg. charges on the DNA polyanion at high ionic strength of the soln., is studied at various surfactant concn. It is demonstrated that the sphere-rod transition of surfactant micelles also affects the T4DNA conformation in an aq. media. The structure of intramol. micelles of DNA-surfactant complexes is studied with the use of the X-ray scattering

technique; it is discovered that surfactant forms regular lamellar or hexagonal structures being complexed with stiff DNA polyanions. The effect of the nonionic surfactant Triton X-100 on the conformational behavior of large T4DNA is examd. through a single-mol. observation with fluorescence microscopy. It is found that T4DNA macromols. exhibit a discrete coil-globule transition with an increase in the Triton X-100 concn. The increase in osmotic pressure in concd. Triton X-100 solns. is considered to be the driving force for the compaction of single T4DNAs.

L6 ANSWER 314 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 117

AB Amphipathic peptides can be useful effectors to enhance gene delivery. However, peptide/DNA complexes usually require addnl. effectors, such as fusogenic lipids, to mediate efficient transfection. Due to weak and/or multiple interactions between the various components of the system, the transfecting complexes are often heterogeneous and unstable in biol. fluids. Accordingly, a hybrid mol. resulting from the covalent coupling of an amphipathic, membrane-disturbing peptide to a lipid moiety might create a stable and efficient peptide-based gene transfer system. The present work describes such a novel hybrid mol., dioleoylmelittin, resulting from the conjugation of dioleoylphosphatidylethanolamine-N-[3-(2-pyridyldithio)propionate] with [Cys1]melittin. Dioleoylmelittin had a lower hemolytic and membrane-disturbing activity than melittin. Size and zeta potential measurements, DNA gel electrophoresis, and electron microscopy showed that dioleoylmelittin, unlike melittin, was able to complex plasmid DNA to form spherical particles with a net pos. charge and a diam. between 50 and 250 nm. These particles, prepd. at an optimal 10/1 dioleoylmelittin/DNA ratio (wt./wt.), mediated efficient transient transfection of reporter genes in cultured mammalian cells including primary cells. The luciferase activity induced by the dioleoylmelittin/**DNA** complex was 5-500-fold higher than that induced by a **cationic lipid/DNA** complex, depending on the **cationic lipid** and the cell-line. Surprisingly, the presence of 10-50% fetal calf serum during dioleoylmelittin-mediated transfection enhanced 1.5-3-fold gene expression. Dioleoylmelittin represents a new class of efficient peptide-based transfection reagents, esp. suited for serum-sensitive cells.

L6 ANSWER 315 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

L6 ANSWER 316 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

L6 ANSWER 317 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

L6 ANSWER 318 OF 420 BIOSIS COPYRIGHT 2001 BIOSIS

AB A commercially available cationic surfactant, dimethyl-dioctadecyl ammonium bromide (DDAB), was used for making lipid vesicles. DDAB easily dissolved in water at 60degreeC and formed lipid vesicles at room temperature. The lipid vesicles showed very low cytotoxicity compared with other cationic surfactants. After the lipid vesicles were mixed with plasmid **DNA** solution, the solution was added to mammalian cells. The addition of a nonionic surfactant (Tween 80) to the **cationic lipid** vesicles at the weight ratio of 1:1 enhanced transfection efficiency. Adding more or less than the optimal amounts of **DNA** and lipid vesicles resulted in decreased transfection efficiency. With the optimal amounts of **DNA** (pCMVbeta) and lipid vesicles, about 90-95% of CHO-K1 and BHK-21C13 cells transiently expressed beta-galactosidase activity 24 h after transfection. By this procedure, stable transformants around 105 cells corresponding to 10% efficiency could be obtained by one batch transfection.

L6 ANSWER 319 OF 420 CAPLUS COPYRIGHT 2001 ACS
AB A method is r[redacted]ted for complexing high concn[redacted]f DNA to cationic lipids
for in vivo use. This complexation method is very reproducible and leads to the formation of highly stable lipid/DNA complexes that are insensitive
to serum. These complexes have attributes highly suitable for in vivo gene transfer. The stable lipid/DNA complex is able to achieve consistently high levels of expression from multiple batches and from different DNA prepns. It has a minimal shelf life of 90 days when stored as a suspension at 4.degree.C. The advantage of the prolonged shelf life is realized in the ability to perform multiple expts. with the same batch of material. His also removes variability when applying multiple administrations.

L6 ANSWER 320 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 118
AB To better understand the structures formed by the interaction of cationic lipids with **DNA**, we undertook a systematic anal. to det. the biophys. characteristics of **cationic lipid:DNA** complexes. Four model cationic lipids with different net cationic charge were found to interact in similar ways with DNA when that interaction was compared in terms of the apparent molar charge ratio of lipid to DNA. When **DNA** was present in charge excess over the **cationic lipid**, the complex carried a net neg. charge as detd. by zeta potential measurements. Under these conditions, some DNA was accessible to ethidium bromide, and free DNA was obsd. in agarose gels and in dextran
d. gradients. Between a lipid:**DNA** charge ratio of 1.25 and 1.5:1, all the **DNA** became complexed to **cationic lipid**, as evidenced by its inaccessibility to EtBr and its complete assocn. with lipid upon agarose gel electrophoresis and d. gradient sepns. These complexes carried a net pos. charge. The transition between neg. and pos. charged complexes a occurred over a very small range of lipid to DNA ratios. Employing a fluorescent lipid probe, the addn. of **DNA** was shown to induce lipid mixing between **cationic lipid**-contg. vesicles. The extent of DNA-induced lipid mixing reached a max. at a charge ratio of about 1.5:1, the point at which all the DNA was involved in a complex and the complex became pos. charged. Together with freeze-fracture electron micrographs of the complexes, these biophys. data have been interpreted in light of the existing models of **cationic lipid:DNA** complexes.

=> d l6 307,314,320 abs ibib

L6 ANSWER 307 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 112
AB Noticeable modifications of in-serum transfection efficiency of dioctadecylamidoglycyl-spermine (DOGS)-DNA complexes are obsd., depending on DNA condensation conditions. The structures of the complexes are studied, keeping in mind the variability of lipid polymorphism, by cryo-TEM and x-ray diffraction. By increasing both pH and ionic strength,
well-organized lamellar structures with a period of 65 .ANG. replace supramicellar aggregates. A relation between the structures and their in-vitro transfection activity is established. Efficiency in the presence
of serum is maintained when a lamellar arrangement is involved.
ACCESSION NUMBER: 1997:358122 CAPLUS
DOCUMENT NUMBER: 127:91923
TITLE: Structure of in-serum transfecting **DNA-cationic lipid** complexes
AUTHOR(S): Boukhnikachvili, T.; Aguerre-Chariol, O.; Airiau, M.; Lesieur, S.; Ollivon, M.; Vacus, J.

CORPORATE SOURCE: Rhone-Poulenc Rorer Gencell, Centre de Recherche de Vitry-Alfortville 13, Quai [redacted]es Guesde, Vitry sur Seine, 94400, Fr.
SOURCE: FEBS Lett. (1997), 409(2), 188-194 QP501.F4
CODEN: FEBLAL; ISSN: 0014-5793
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

L6 ANSWER 314 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 117
AB Amphipathic peptides can be useful effectors to enhance gene delivery. However, peptide/DNA complexes usually require addnl. effectors, such as fusogenic lipids, to mediate efficient transfection. Due to weak and/or multiple interactions between the various components of the system, the transfecting complexes are often heterogeneous and unstable in biol. fluids. Accordingly, a hybrid mol. resulting from the covalent coupling of an amphipathic, membrane-disturbing peptide to a lipid moiety might create a stable and efficient peptide-based gene transfer system. The present work describes such a novel hybrid mol., dioleoylmelittin, resulting from the conjugation of dioleoylphosphatidylethanolamine-N-[3-(2-pyridyldithio)propionate] with [Cys1]melittin. Dioleoylmelittin had a lower hemolytic and membrane-disturbing activity than melittin. Size and zeta potential measurements, DNA gel electrophoresis, and electron microscopy showed that dioleoylmelittin, unlike melittin, was able to complex plasmid DNA to form spherical particles with a net pos. charge and a diam. between 50 and 250 nm. These particles, prepd. at an optimal 10/1 dioleoylmelittin/DNA ratio (wt./wt.), mediated efficient transient transfection of reporter genes in cultured mammalian cells including primary cells. The luciferase activity induced by the dioleoylmelittin/**DNA** complex was 5-500-fold higher than that induced by a **cationic lipid/DNA** complex, depending on the **cationic lipid** and the cell-line. Surprisingly, the presence of 10-50% fetal calf serum during dioleoylmelittin-mediated transfection enhanced 1.5-3-fold gene expression. Dioleoylmelittin represents a new class of efficient peptide-based transfection reagents, esp. suited for serum-sensitive cells.
ACCESSION NUMBER: 1997:127037 CAPLUS
DOCUMENT NUMBER: 126:113857
TITLE: Dioleoylmelittin as a Novel Serum-Insensitive Reagent for Efficient Transfection of Mammalian Cells
AUTHOR(S): Legendre, J. Y.; Trzeciak, A.; Bohrmann, B.; Deuschle, U.; Kitas, E.; Supersaxo, A.
CORPORATE SOURCE: Preclinical Research and Development, F. Hoffmann-La Roche AG, Basel, CH-4070, Switz.
SOURCE: Bioconjugate Chem. (1997), 8(1), 57-63 QP517.B49.B5C
CODEN: BCCHES; ISSN: 1043-1802
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

L6 ANSWER 320 OF 420 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 118
AB To better understand the structures formed by the interaction of cationic lipids with **DNA**, we undertook a systematic anal. to det. the biophys. characteristics of **cationic lipid:DNA** complexes. Four model cationic lipids with different net cationic charge were found to interact in similar ways with DNA when that interaction was compared in terms of the apparent molar charge ratio of lipid to DNA. When **DNA** was present in charge excess over the **cationic lipid**, the complex carried a net neg. charge as detd. by zeta potential measurements. Under these conditions, some DNA was accessible to ethidium bromide, and free DNA was obsd. in agarose gels and in dextran

d. gradients. Between a lipid:**DNA** charge ratio of 1.25 and 1.5:1, all the **DNA** became complexed to **cationic lipid**, as evidenced by its inaccessibility to EtBr and its complete assocn. with lipid upon agarose gel electrophoresis and d. gradient sepns. These complexes carried a net pos. charge. The transition between neg. and pos. charged complexes a occurred over a very small range of lipid to DNA ratios. Employing a fluorescent lipid probe, the addn. of **DNA** was shown to induce lipid mixing between **cationic lipid**-contg. vesicles. The extent of DNA-induced lipid mixing reached a max. at a charge ratio of about 1.5:1, the point at which all the DNA was involved in a complex and the complex became pos. charged. Together with freeze-fracture electron micrographs of the complexes, these biophys. data have been interpreted in light of the existing models of **cationic lipid:DNA** complexes.

QD1, B51

ACCESSION NUMBER: 1997:177510 CAPLUS
DOCUMENT NUMBER: 126:273932
TITLE: Biophysical characterization of **cationic lipid:DNA** complexes
AUTHOR(S): Eastman, S. J.; Siegel, C.; Tousignant, J.; Smith, A. E.; Cheng, S. H.; Scheule, R. K.
CORPORATE SOURCE: Genzyme Corporation, One Mountain Road, Framingham, MA, 01701-9322, USA
SOURCE: Biochim. Biophys. Acta (1997), 1325(1), 41-62
CODEN: BBACAQ; ISSN: 0006-3002
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

=> d his

(FILE 'HOME' ENTERED AT 14:59:50 ON 19 MAR 2001)

FILE 'CAPLUS, BIOSIS' ENTERED AT 15:00:36 ON 19 MAR 2001
L1 1214 S CATIONIC (A) LIPID
L2 972 S CATIONIC (A) LIPOSOME
L3 972 S CATIONIC(A)LIPOSOME
L4 568 S DNA (S) L1
L5 366 S DNA (S) L2
L6 420 DUP REMOVE L4 (148 DUPLICATES REMOVED)
L7 1 S (DIMER? OR OLIGOMER?) (S) L6
L8 27 S (LINK? OR BIND?) (S) L6
L9 0 S (THIOL? OR DISULFIDE BRIDGE? OR CYSTEINE) (S) L6

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	276.93	277.08
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-32.34	-32.34